Characterization of and Biological Nitrogen Removal from Landfill Leachate

by

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ABSTRACT

Leachates were collected from the closed Ma Yau Tong Central (MYT) Landfill and the operating Pillar Point Valley (PPV) Landfill. Their physico-chemical properties were analyzed every two months from April 1994 to February 1995. Leachate from the closed MYT site had lower concentration of biochemical oxygen demand (BOD) (18.7 - 72.8 mg/L) and biochemical oxygen demand:chemical oxygen demand (BOD:COD) ratio (0.02 - 0.11). BOD of the operating PPV landfill decreased during the study period, from 664 to 38.1 mg/L while COD remained at high level (642 to 971 mg/L). On the other hand, ammoniacal-N (NH_x-N) concentration in the leachate from the closed MYT Landfill was higher than that of the operating PPV Landfill. Average concentrations of ammoniacal-N in MYT and PPV leachates were 788 and 656 mg/L respectively. Ammonia, the major pollutant in leachate, must be removed before the leachate is discharged to the environment. Leachates from both landfills had low concentrations of phosphorus and heavy metals (cadmium, chromium, copper, nickel and lead) except iron, manganese and zinc. MYT leachate showed a better correlation with cumulative rainfall for chemical parameters such as total solids (TS), electrical conductivity (EC), NH_x-N, total Kjeldahl phosphorus (TKP) and orthophosphate-P (PO_4^{3-}-P) ($r > 0.80$ and $P < 0.05$). However, the correlation for the operating PPV landfill was poor. Microbiological study of the leachates showed that population of bacteria (10^4 - 10^5 CFU/mL) was much higher than fungi (43 - 178 CFU/mL). Populations of carbohydrate-, protein- and lipid-utilizing bacteria followed the same sequence as the concentrations of the corresponding substrates in the leachates.

Toxicity effects of leachates collected from MYT and PPV Landfill in June and December 1994 were assessed by *Photobacterim phosphoreum* (bacterium), *Chlorella pyrenoidosa* (alga), *Moina macrocopa* (crustacean) and *Brachydanio rerio* (fish). Different species exhibited differential sensitivities to the leachates. Growth of *C. pyrenoidosa* was not affected by the leachates. Nutrients provided by the leachates stimulated algal growth at low leachate concentrations (3, 6 and 12%). EC50 (5-, 15- and 30-min) of Microtox test of MYT leachates were higher than 50% for samples of both months. For PPV leachate, values of EC50 changed with time of exposure. On the other hand, *B. rerio* was highly sensitive to MYT and PPV leachates. All the fish died at 4 - 8% leachate. Forty eight-hour LC50 of *M. macrocopa* test for June and December samples from MYT were 15.5 and 16.3% respectively, while those for PPV leachate were 16.4 and 19.4% respectively. Due to differential sensitivities of organisms to leachate, it is recommended that more than two organisms must be used to assess leachate toxicity and *M. macrocopa* appears to be the best test subject for estimating leachate toxicity.

Efficiency of treating MYT leachate by nitrification was investigated with a bench scale, suspended growth, continuous flow system under different phosphorus levels, hydraulic retention times (HRT) and organic carbon dosages. Systems with influent containing 2.36, 5 and 10 mg/L PO_4^{3-} produced effluent of similar quality. Low phosphorus level of leachate was not a limiting factor for ammonia removal and nitrate production. Increase of HRT from 1 to 2 days resulted in increase of solid concentration and efficiency of ammonia removal. However, further increase of HRT to 4 days did not improve the efficiency to a greater extent. Ammonia removal of the system with influent BOD of 1000 mg/L were much higher than systems with BOD of

42.5 and 100 mg/L. Improvement of sludge settleability was also observed. Effluent with lower nitrate concentration was produced. Ammonia removal efficiency dropped once the system was fed with unspiked leachate influent.

Effluent produced from the nitrification system without phosphate and carbon amendment and HRT of two days was further treated by a denitrification system. The denitrification systems were a continuous flow system with HRT of 1, 2 and 4 days. COD of nitrified leachate increased significantly, and COD:NO_3^--N ratio was 5.87:1. The organic carbon may not be readily usable and only 10 - 30% of nitrate was removed. System with longer HRT showed higher removal efficiency. Modifications are necessary for the improvement of nitrification and denitrification efficiency, such as the use of denitrification-nitrification system, the addition of organic carbon or use of attached growth system.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
BOD	5-day biochemical oxygen demand
CFU	Colony forming unit
COD	Chemical oxygen demand
DO	Dissolved oxygen
DS	Dissolved solids
EC	Electrical conductivity
EC50	Median effective concentration
F:M	Food to microorganisms ratio
HRT	Hydraulic retention time
LC50	Median lethal concentration
LSD	Least significant difference
MLSS	Mixed liquor suspended solids
MLVSS	Mixed liquor volatile suspended solids
MPN	Most probable number
MW	Molecular weight
NH_3-N	Ammonia-nitrogen
NH_x-N	Ammoniacal-nitrogen
NO_2^--N	Nitrite-nitrogen
NO_3^--N	Nitrate-nitrogen
NO_x-N	Oxidized-nitrogen
PO_4^{3-}-P	Orthophosphate-phosphorus
RBC	Rotating biological contactor
RO	Reverse osmosis
SBR	Sequencing batch reactor
SD	Standard deviation
SRT	Sludge retention time
SS	Suspended solids
TKN	Total Kjeldahl nitrogen
TKP	Total Kjeldahl phosphorus
TOC	Total organic carbon

TS	Total solids
UASB	Upflow anaerobic sludge blanket

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1 INTRODUCTION

1.1 LANDFILLING IN HONG KONG

Large quantities of wastes are produced every day in Hong Kong which has a population of more than six million. In 1994, an average of three million tonnes of solid wastes was produced every day (Environmental Protection Department, 1995). Solid waste arisings have increased roughly in line with the increase in Gross Domestic Product and it is forecast to rise to 4.8 million tonnes by the year 2006. At present, incinerators are being phased out because of air pollution problem and the difficulty in siting new incinerator. Landfilling has become the sole waste disposal method in Hong Kong. There are now 13 'old' landfills in the territory and only Pillar Point Valley Landfill is still under operation (Table 1.1). New disposal and treatment facilities are being explored to meet the increasing waste arisings.

As the existing landfills are exhausted, three big strategic landfills, namely the WENT (West New Territories) Landfill, SENT (South East New Territories) Landfill and NENT (North East New Territories) Landfill have been constructed and commissioned to receive wastes for their ultimate disposal. These modern landfills are operated according to high environmental standards. Impervious bottom lining systems are incorporated so that leachate is contained, collected and treated. Landfill gas is extracted to generate electricity for power plant on site and for exporting off site for other users.

In contrast to the strategic landfills, the 13 old landfills were constructed at time when landfill technology was less developed and environmental requirement was less strict. They are inadequately designed and have few measures for controlling pollution problems. Landfill gas becomes a potential hazard at both the

Table 1.1 Landfills in Hong Kong (Environmental Protection Department, 1996).

Landfill	Area (hectare)	Capacity (million tonnes)	Year of closure
Ngau Tam Mei	2.0	0.03	1975
Ngau Chi Wan	13.5	0.7	1977
Gin Drinkers Bay	29.0	3.5	1979
Ma Tso Lung	2.0	0.2	1979
Sai Tso Wan	14.0	1.3	1980
Ma Yau Tong (West)	6.6	0.6	1981
Siu Lang Shui	11.7	1.2	1983
Ma Yau Tong (Central)	9.9	1.0	1986
Jordan Valley	6.5	1.5	1990
Tseung Kwan O II/III	35.0	12.6	1994
Tseung Kwan O I	68.0	15.2	1995
Pillar Point Valley	53.0	13.0	operating
Shuen Wan	50.0	14.3	1995

landfill and adjacent areas. Landfill leachate causes water pollution problems and damages the surrounding sewers. In order to alleviate the environmental impacts of old landfills, restoration work will be progressed in phases. The first restoration contract commenced in early 1996 and may last for up to 30 years until the site is safe for other land uses. Restoration program includes installation of landfill gas abstraction system or venting trench, leachate treatment and site landscaping (Environmental Protection Department, 1995).

1.2 Generation of Landfill Leachate

Landfill leachate is generated when water percolates through the wastes buried in the landfill. Water takes up the organic and inorganic products of hydrolysis and biological degradation of solid wastes as well as soluble salts and particulate matters in the wastes (Fig. 1.1). An understanding of the stabilization process in landfill is very helpful in predicting leachate quality. Generally, stabilization process in landfill can be divided into 5 stages according to the metabolic processes which take place in the wastes (Andreottola and Cannas, 1992; Christensen and Kjeldsen, 1989; Harmsen, 1983).

Phase I is a short aerobic period immediately after deposition of wastes. Oxygen is trapped in the freshly buried wastes and is also supplied by infiltrating rainwater. Easily degradable organic matter is aerobically decomposed. Only small amount of leachate is formed during Phase I.

Immediately after aerobic phase, anaerobic phase develops (Phase II). The activities of the fermentation and acetogenic bacteria result in rapid generation of volatile fatty acids, amino acids, other low molecular weight compounds and gases

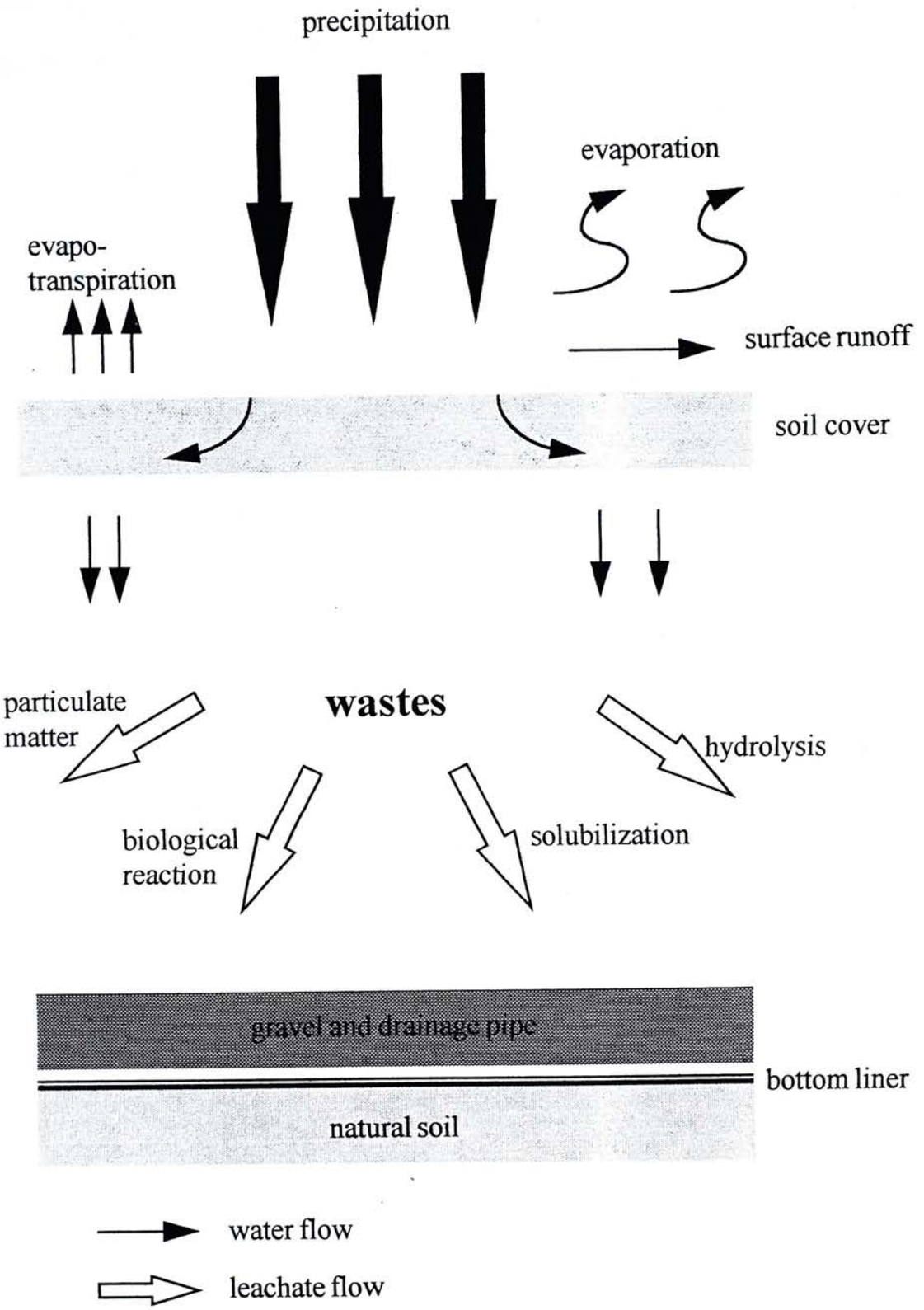


Fig. 1.1 Formation of landfill leachate (Lema *et al.*, 1988).

such as carbon dioxide and hydrogen. Hydrolysis and fermentation of proteinaceous compounds produce large amount of ammonia. This results in the formation of leachate with low pH (about 5 - 6), high organic strength and high ammonia concentration (500 - 1000 mg/L). More than 90 - 95% of total organic carbon (TOC) in acetogenic leachate is volatile fatty acids (Harmsen, 1983; Johansen and Carlson, 1976). This leachate has very high biochemical oxygen demand (BOD) to chemical oxygen demand (COD) ratio, ranging between 0.4 - 0.8 (Oman and Hynning, 1993), which indicates that most of the organics present in the leachate are biodegradable.

Phase III begins when there is a slow growth of methanogenic bacteria. Concentration of volatile fatty acids decreases to very low level as the methanogenic bacteria convert fatty acids into methane and carbon dioxide. This results in increasing pH and alkalinity of leachate. Values of TOC, COD and BOD decrease. BOD:COD ratio also decreases to below 0.1 at this stage. Ammonia is being released and remains at high level because it is not converted to any other forms in the anaerobic environment.

Phase IV is reached when the production rate of methane becomes stabilized, i.e. 50 - 65% of landfill gases. The high rate of methane formation maintains the low concentration of volatile fatty acid. As a result, leachate produced has neutral pH, relatively low BOD, and low ratio of BOD:COD. Large amount of ammonia is released continuously.

Phase V is reached when refractory organic carbon represents majority of organics in the leachate and the methane production rate becomes very low.

1.3 Composition of Landfill Leachate

Several factors affect the composition of leachate and its production. These include waste composition, operation of fill, climate, site hydrogeology, age of fill (more accurately, the degree of stabilization) and landfill conditions such as moisture and temperature (Chian and DeWalle, 1976; Cyr *et al.*, 1987; Johansen and Carlson, 1976; Kennedy *et al.*, 1988). Different regions of a landfill may have wastes highly varied in age and composition and leachate formed may be a mixture of liquor from different phases.

According to the stabilization process, landfill leachate can be classified into two major categories — acetogenic and methanogenic leachates. Some parameters, such as pH, organic contents and concentrations of ions such as sulfate, calcium, magnesium and metals would show a great difference in acetogenic and methanogenic leachate. However, others, such as ammonia, nitrate, phosphate, chloride, sodium, potassium and some heavy metals such as cadmium, nickel, lead, chromium, copper, are similar for acetogenic and methanogenic leachate (Ehrig, 1989). Leachate may have characteristics intermediate between these two major types, depending on the degree of stabilization in the landfill (Table 1.2)

Free volatile fatty acids represent the majority of organics in leachate from freshly buried wastes. Acetic acid, propionic acid, butyric acid and valeric acid are those most commonly found in leachate (Hoeks and Borst, 1982). Concentration of acids decreases considerably as the landfill ages (Chian, 1977), and this can be reflected by the rapid decrease in BOD:COD ratio of the leachate when the landfill stabilizes. The second largest group of organics found in leachate from young landfills is fulvic-like material with intermediate molecular weight (MW 500 -

Table 1.2 Composition of acetogenic and methanogenic leachates.

Landfill	Age (years)	pH	BOD: COD	BOD (mg/L)	COD (mg/L)	TOC (mg/L)	Carboxy-lic acid (mg/L)	TKN (mg/L)	NH_x-N (mg/L)	TKP (mg/L)	PO_4^{3-}-P (mg/L)
Acetogenic leachate											
Sand Farm (UK)[a]	-	6.73	0.76	7,800	10,200	-	1,090	-	528	-	-
Britannia (Canada)[b]	11	6.86	0.58	5,340	9,254	-	4,581	-	196	-	-
Sandtown (Delaware)[c]	-	5.4-5.5	0.81	19,000-31,500	14,000-36,000	-	-	160-580	650-780	-	< 0.1
Ambt-Delden (Netherland)[d]	9	5.7	0.50	30,000	60,000	20,000	-	-	-	-	-
Yggeseth (Norway)[e]	9	5.9	0.56	5,250	9,425	1,700	-	250	227	-	7.7
Cedar Hill (USA)[e]	10	5.4	0.63	24,500	38,800	-	-	-	-	-	11.3
Methanogenic leachate											
Pitsea (UK)[f]	> 60	8.0-8.5	0.1	80-250	850-1,350	200-650	20	-	200-600	-	0.2
Moyer (Pennsylvinia)[g]	> 50	7.2-7.7	0.07	15-38	322-385	44-92	-	-	148-160	-	-
Wijster (Netherland)[d]	2	7.0	0.01	50	7,000	2,100	-	-	-	-	-
Intermediate between acetogenic and methanogenic leachates											
Beare Road (Canada)[h]	18	6.58	0.50	1,870	1,070	-	1,480	75	10	0.15	-
Brock North (Canada)[h]	8	6.35	0.30	9,750	1,100	-	1,170	42	36	36	-
Bailey Road (Canada)[i]	-	7.9	0.32	1,167	373	-	-	-	71	-	-

a Robinson, 1987
b Laughlin *et al.*, 1992
c Gaudy *et al.*, 1986
d Harmsen, 1983
e Johansen and Carlson, 1976
f Knox, 1985
g Spengel and Dzombak, 1991
h Henry *et al.*, 1987
i Kelly, 1987

10,000). This group of compounds is characterized by high density of carboxyl and aromatic hydroxyl groups. A small percentage of organics exists as a high molecular weight humic-carbohydrate-like complex (MW > 10,000) with a significant amount hydrolyzable amino acid group that originates from bacteria excretion (Chian and DeWalle, 1977). As the age of fill increases, humic-carbohydrate-like materials decreases more rapidly than the fulvic-like materials. This is because of preferential microbial degradation and conversion of humic materials into fulvic-like materials by chemical processes (Chian and DeWalle, 1977).

Organic compounds decrease more rapidly than inorganic compounds because of their biodegradable nature. The reduction of organics involves both biological degradation and washout, while the decrease of inorganics involves only physical washout by infiltration water.

Owing to the hydrolysis and fermentation of the proteinaceous fraction of the biodegradable substrate, ammonia concentration in leachate is usually very high and contributes to 60 - 90% of the total nitrogen compounds. Unlike carbon compounds which are converted to carbon dioxide and methane, the only route for nitrogen to leave a landfill is by dissolving in leachate as ammonium. Ammonium would not be readily oxidized to nitrite or nitrate due to the anaerobic condition in landfill. Many 'old' leachates contain low concentration of organic compounds but still very high level of ammonia for a long period of time.

Low concentration of phosphorus is found in acetogenic and methanogenic leachates (Table 1.2). This is a typical characteristic of leachates from all types of landfills, irrespective of their ages.

Unlike other inorganic contents, there is a remarkable difference in heavy

metal content between acetogenic and methanogenic leachates (Table 1.3). During the acetogenic stage, the metals are more soluble because of the low pH and the ability of forming complex with free volatile acids. In the methanogenic stage, fatty acids are converted to methane and pH rises. Solubility of metals greatly decreases and concentration of metals drops drastically. Sulfide formed in the anaerobic phase also precipitates out the metals.

Short-term quality of leachate is affected greatly by the quantity of leachate formed at a given period of time, which is in turn dependent upon the volume of water entering the landfill. The degree of correlation is very site specific and depends on fill conditions. One may consider that strength of leachate would decrease when water flow in landfill increases, but this is not always true for every landfill. Concentrations of TOC, COD, Fe and Mn in leachate from a landfill in Seattle, decreased rapidly with increasing of flow in the old area, whereas leachate quality was almost independent of flow in the new filled area (Ragle *et al.*, 1995). Time lag of leachate appearance was 4 days and 30 days after rainfall for old area and new area respectively. Higher degree of channelization in old area resulted in shorter time lag. Leachate composition was found to correlate with 7-day cumulative rainfall for an 11-year-old Gin Drinkers Bay Landfill but with 14-day cumulative rainfall for a 3.5-year-old co-disposal test cell in Tseung Kwan O Landfill in Hong Kong (Chu *et al.*, 1994).

When comparing the composition of landfill leachate with sewage, it could found that leachate is a higher strength wastewater both in terms of organic and inorganic contents (Table 1.4). Organic strength of young leachate is about 35 times as high as that of sewage. Much higher level of ammonia, chloride, sodium, magnesium, potassium and calcium could be found in leachate. High concentrations

Table 1.3 Heavy metal concentrations (mg/L) in landfill leachate.

Landfill	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Acetogenic leachate								
Keele Valley[a] (Canada)	0.10	-	0.19	1070	-	1.08	-	5.04
Ambt-Delden (Netherland)[b]	0.01	-	0.65	1120	53	1.04	0.17	54
Methanogenic leachate								
Tseung Kwan O (co-disposal test cell) (HK)[c]	< 0.01	0.03-0.15	< 0.05	1.14-3.25	0.05-0.24	0.07-0.18	0.03-0.12	0.24-2.55
Gin Drinkers Bay (HK)[c]	< 0.02	< 0.05	0.09	10	0.50	0.04	0.10	0.16
Pitsea (UK)[d]	< 0.02	< 0.05	0.09	10	0.50	0.04	0.10	0.16

a Henry *et al.*, 1987
b Harmsen, 1983
c Chu *et al.*, 1994
d Robinson, 1987

Table 1.4 Comparison of composition of domestic sewage and landfill leachate (Harrington and Maris, 1986).

Parameters	Leachate		Domestic
	fresh wastes	aged wastes	sewage
pH	6.2	7.5	7.5
COD	24000	700	700
BOD	13600	70	400
TOC	8000	400	240
Volatile fatty acids (as C)	6000	< 5	< 40
NH_x-N	600	260	46
NO_x-N	< 0.5	7.5	< 0.5
PO_4^{3-}-P	0.7	0.5	14.0
Cl	1300	1400	120
Na	960	880	100
Mg	250	130	4.5
K	780	340	20
Ca	1820	200	110
Cr	0.56	0.07	0.005
Mn	26.5	1.7	0.07
Fe	540	10	0.03
Zn	21.5	0.20	0.16

All in mg/L except pH.

of calcium, magnesium and iron render leachate treatment works more susceptible to scaling and clogging. Carbonate, sulfite and hydroxides of these ions frequently cause operation problems of leachate treatment (Keenan *et al.*, 1984; Kennedy *et al.*, 1988; Knox, 1985).

As the fill stabilizes, the inorganic concentrations of leachate only decrease to a very small extent. Leachate produced at this period has similar COD value to sewage but its degradability, as reflected by the BOD:COD ratio, is much lower than that of sewage (Harrington and Maris, 1986).

Leachate is regarded as a more difficult waste when compared with sewage. Leachate contains much higher concentration of pollutants. In addition to the diurnal and seasonal fluctuation, alternation of leachate quality as the landfill ages is a unique problem confronting many leachate treatment plants.

1.4 Toxicity of Landfill Leachate

Leachate contains a complex of inorganic and organic constituents. It is not only deleterious the quality of ground and surface water but also toxic to biotic components of the aquatic and terrestrial ecosystems. Although the toxicity effects of leachate are not studied as extensively as chemical properties, experiments have already demonstrated its toxicity to organisms from different trophic levels.

As leachate is usually discharged to the aquatic environment, aquatic organisms are mostly used to assess leachate toxicity. Leachate from a landfill containing about 40% household and 60% industrial waste was tested with different organisms (Ploktin and Ram, 1984). Five-min EC50 of Microtox test of raw and filtered leachate were 14% and 17% respectively. Growth of an alga, *Selenastrum*

capriconutum, was inhibited to 50% when leachate concentration was 1 - 10%. Forty eight-hour EC50 of *Daphnia magna* (water flea) was 62 - 66% of leachate.

Leachate from a co-disposal test cell of a landfill (Tseung Kwan O) and a municipal landfill (Gin Drinkers Bay) in Hong Kong exhibited different toxicity effects on the growth of 4 algal species, *Chlorella pyrenoidosa*, *C. vulgaris*, *Scenedesmus* sp., *Dunaliella tertiolecta* (Cheung *et al.*, 1993). Leachate from the younger co-disposal site was more toxic to the algae. 96-h EC50 of *D. tertiolecta* was < 5%, *C. vulgaris* 7%, *Senedesmus* sp. 20% and *C. pyrenoidosa* 23%. 96-h EC50 of leachate from the closed landfill for the four algal species. was 21%, 33%, > 50% and > 50% respectively. The toxicity of the leachate from younger co-disposal site was higher than that from the closed landfill because of higher concentration of free ammonia and organic substances. The indirect effects of pH and color also contributed to leachate toxicity.

Acute toxicity of leachate to rainbow trout was examined (Cameron and Koch, 1980). Ninety six-hour LC50 was as low as 5.6% of leachate. Unionized ammonia, hydrogen ion, tannin and copper were responsible to the toxicity. When the leachate was treated by peat filtration or lime coagulation followed by peat filtration, toxicity effect was eliminated.

In addition to acute toxicity, long term effect of leachate on organisms was also studied. The acute and sublethal toxicities of leachate from Burn Beg Sanitary Landfill in Canada were examined using rainbow trout (*Salmo gairdneri*) (McBride *et al.*, 1979). Ninety six-hour EC50 was 5.8 - 7.5% of leachate. When trout was exposed to sublethal concentration of leachate (5%), the cortisol was increased which indicated fish was under stress condition. Cortisol remained at elevated level even

after being returned to normal environment. Interrenal nuclear diameters, which associated with increased activities of nuclei, were considerably greater after 2 days of exposure, and degree of size change increased with time of exposure. These histological change might related to fish adaptation to exposure to leachate.

Toxicity of leachate was also assessed using higher plants. Both seed germination and root growth of *Brassica chinensis* and *Cynodon dactylon* were retarded by leachate with concentration at or greater than 25% (Tong and Wong, 1984).

Due to the co-disposal of hazardous wastes with municipal refuse in landfills and the uncontrolled manner of disposal in the past, the potential mutagenicity of leachate has become a problem of increasing concern. Leachates from four different landfills, including a closed municipal landfill, an operating municipal landfill, a closed co-disposal landfill of municipal and hazardous wastes and an operating co-disposal landfill were tested (Schrab *et al.*, 1993). Leachate from the operating co-disposal landfill induced positive response in *Bacillus subtilis* DNA repair bioassay. Other three leachates induced positive response in the diploid *Aspergillus nidulans* chromosome damage bioassay. Due to acute toxicity of the leachates to *Salmonalla*, mutagenic effect could not be assessed by the Ames test. All of the four samples were found to be acutely toxic by Microtox test. Leachates from either municipal landfill or hazardous waste co-disposal landfill were acutely and chronically toxic. In another study, a resin concentrated leachate, which was collected from a municipal domestic waste landfill, exhibited a positive result in the Ames test (Omura *et al.*, 1991).

1.5 TREATMENT OF LANDFILL LEACHATE

Due to the hazardous effects of landfill leachate on the environment, collection and treatment prior to discharge are necessary. The major operational problem is the change in leachate properties as the age of landfill increases. A notable change is a decrease in BOD which results in the reduction of degradability. New pollution control regulations would also alter the demand on effluent quality and hence the degree of treatment. The most suitable method for leachate treatment varies with time. A number of treatment methods have been examined for leachates from different landfills. However, no single technique is suitable for all applications. Experience from one site may not be transferable to another. It may be necessary that each landfill should be engineered separately. Choice of a treatment scheme at a specific site will depend on the chemical nature of leachate, degree of treatment needed, effluent discharge alternative, equipment and personnel available on the site. The optimum design should be robust and flexible to allow modification of treatment method as the landfill ages or effluent standards tighten. For landfill which receives no hazardous wastes and is producing acetogenic leachate, system that can handle very high organic loading should be employed. When methanogenic leachate is treated, the system must be able to remove high percentage of refractory organics and high concentration of ammonia.

Various methods have been tested for the treatment of leachate, either in bench or field scale. In general, leachate treatment methods can be grouped into physico-chemical treatment, biological treatment, co-treatment with municipal wastewater, recirculation and irrigation. Their operational problems in treating leachate and their efficiency, especially in the removal of COD and ammonia, will be

discussed.

1.5.1 Physico-chemical treatment

Physico-chemical method is usually employed to treat low organic strength leachate or included as a pre- or post-treatment step for conventional biological treatment. Precipitation, coagulation, flocculation, oxidation, activated carbon adsorption and reverse osmosis are widely examined. The feasibility of leachate treatment by evaporation (Cossu *et al.*, 1992), radiation (Britz, 1995) or electrical oxidation (Chiang *et al.*, 1995) have also be investigated.

When compared with biological methods, physico-chemical processes are faster in start-up, shorter in reaction time which results in smaller plant size, less sensitive to environment conditions and is more flexible in treating leachate with fluctuating properties. The major disadvantages are the high cost of chemicals and the larger volume of sludge produced.

1.5.1.1 Coagulation/Flocculation/Precipitation

Coagulation, flocculation and precipitation are individual processes, but are often combined into a single overall treatment stage. During coagulation, colloidal particles, which have dimension of 1 nm - 1 μm and large specific surface, are agglomerated into larger particle due to the action of coagulants such as Al^{3+}, Fe^{3+} and polymers of organic compound. If flocculant such as alum or polyelectrolyte is added, particles coagulate and settle out at a faster rate. Coagulation and flocculation can remove compounds which are responsible for turbidity and color. Dissolved organic compounds with dimension about 1 nm can be efficiently removed by

flocculation (Cossu *et al.*, 1992). Precipitation is used to describe the phase that immediately follows flocculation, and also to the formation of insoluble compounds by the addition of reagent(s). Precipitation of heavy metals by forming of metal hydroxides or sulfides is an example. The particles formed during coagulation/flocculation/precipitation are then allowed to settle out and a clarified effluent is produced.

Lime, $Ca(OH)_2$, is one of the most commonly used chemicals for precipitation/coagulation. Although lime is effective in color and iron removal, the ability of COD removal is limiting; only 20 - 30% of COD could be removed when high dose of lime was employed (Boyle *et al.*, 1974; Chian and DeWalle, 1976; Diamadopoulos, 1994; Kinman and Nutini, 1992; Knox, 1983). Lime precipitation was most effective in removing organic matter with MW > 50,000 which is not the predominant organics found in landfill leachate (Qasim and Chiang, 1994). However, as addition of lime increases the pH of leachate, it can facilitate the removal of ammonia if stripping is carried out subsequently (Cheung *et al.*, in press).

Alum is another coagulant/flocculant commonly used for leachate treatment. The COD removal efficiency of alum varied, from 5 to 45% (Qasim and Chiang, 1994). Performance was determined by pH, and optimum removal was obtained at acidic pH (4.75) (Diamadopoulos, 1994). The sludge formed by alum was found to have poorer settlement than the limed sludge.

Efficiency of ferric chloride ($FeCl_3$) for removing iron and COD was found to be maximum at neutral pH (7) and decreased with decreasing pH (Boyle *et al.*, 1974). However, the settleability of the sludge was much lower than that of limed and alum-sludge (Diamadopoulos, 1994).

In general, precipitation/coagulation/flocculation by adding chemicals has a low efficiency in organic removal (< 30%). Removal of color, suspended solids and metals are good, although high sludge yield would mean further treatment for residue is needed.

1.5.1.2 Oxidation

Unlike other physico-chemical methods, oxidation destroys organic matter and produces a smaller volume of sludge. Oxidation may provide a final solution of treatment. Chemical oxidizing agents commonly used in wastewater treatment include chlorine, calcium hypochlorite, potassium permanganate, ozone and hydrogen peroxide.

In addition to its oxidizing ability, chlorine is sometimes employed as a disinfectant. Although it exhibits good color removal capacity and can remove 95% of iron at a dosage of 400 mg/L (Boyle *et al.*, 1974), it is not effective for ammonia and COD. Dosage as high as 1200 mg/L could only remove 25% COD of leachate which had an initial COD level of 340 mg/L (Boyle *et al.*, 1974). Residue of chlorine may pose another pollution problem to the receiving water.

Calcium hypochlorite also exhibits good efficiency in removing iron and color. However, very high dosage (8000 mg/L) was required in order to obtain a reasonable COD removal of 48% (Boyle *et al.*, 1974). Performance of potassium permanganate is much poorer. Ten thousand mg/L $KMnO_4$ could only remove 20% COD present in the leachate (Boyle *et al.*, 1974).

Ozone and hydrogen peroxide are regarded as better oxidizing agents than the above-mentioned chemicals as they do not produce toxic residues. The highly active

ozone can break down compounds that are resistant to other chemicals or biological oxidation. It is also possible that refractory compounds be oxidized to become more biodegradable. However, H_2O_2-treated leachate had settling problem because of gas formation for a prolonged period of time (Kinman and Nutini, 1992).

1.5.1.3 Activated carbon adsorption

Adsorption by activated carbon is the most common method for removing organic pollutants in wastewater treatment. Activated carbon exhibited the best adsorption ability for compounds with MW 100 - 10,000, such as fulvic acid which is one of the most abundant organic compound in leachate (Chian and DeWalle, 1974). As leachates can differ in their organic profiles to various extents, the performance of activated carbon showed a great variation (Qasim and Chiang, 1994). Poor performance would be observed if there are large amount of polar and high molecular weight (MW > 50,000) organic compounds which activated carbon adsorbs poorly (Chian and DeWalle, 1974). Generally, increase in activated carbon dosage increased the COD removal up to a certain limit (Diamadopoulos, 1994). Increase the dosage could decrease the time for reaching equilibrium (Boyle *et al.*, 1974). Pretreatment with lime prior to adsorption enhanced COD removal (Uloth and Mavinic, 1977). In addition, activated carbon is effective in removing color and metals.

Experiments had been conducted to combine biological treatment method with activated carbon adsorption (Imai *et al.*, 1993 and 1995; Mcshane *et al.*, 1988; Ying *et al.*, 1987). A suspended growth biological culture was supplemented with powdered activated carbon. The rationale of this process is to protect the biological system by adsorbing the toxic compounds in the leachate. This may be especially

useful to leachate from hazardous wastes. Concentrations of phenol, benzoic acid, polychlorinated biphenyls (Aroclor), trichlorobenzenes, hexachlorocyclopentadiene, trichlorophenol, bisoxymethylene sulfite (Endosulfan) and perchloropentacyclo-decane (Mirex) in leachate from landfill containing chemical wastes were decreased significantly in activated carbon added reactor when compared with a conventional sequencing batch reactor (SBR) (Ying *et al.*, 1987). SBR with 3000 mg/L activated carbon could effectively remove halogenated organics to undetectable level. In another study, activated carbon added activated sludge system produced effluent with concentrations of acetone, methy ethyl ketone and tetrahydrofuran concentration 10-fold lower than the activated sludge system alone (Mcshane *et al.* 1988). Adsorbed carbon would eventually be degraded by microorganisms given the long retention time in the adsorbed state. The use of bioactivated carbon also facilitates the maintenance of high sludge age. Adsorption increases sludge density and compaction, and thus resulted in better settleability than conventional suspended growth system (Mcshane *et al.*, 1988). Improvement of carbon removal after addition of activated carbon reported from different studies ranged from 10 to 40% (Imai *et al.*, 1993; Mcshane *et al.*, 1988).

1.5.1.4 Ammonia stripping

Ammonia will become the predominant pollutant in leachate as the landfill ages. Although chlorination and ion exchange could remove ammonia, stripping is the most commonly studied one. In ammonia stripping, air is pumped into the wastewater to bring the ammonia gas, which is in equilibrium with ammonium ion, to the atmosphere.

$$NH_4^+{}_{(aq)} + OH^-{}_{(aq)} \rightleftharpoons NH_{3(g)} + H_2O_{(l)} \qquad \text{(Eq 1.1)}$$

Although it is possible to remove ammonia by air stripping alone, lime is usually employed before stripping, i.e. lime stripping. Lime is added to raise the pH to 10.5 - 11.5 so that the equilibrium is shifted to the formation of gaseous ammonia. After aerating for 24 h at rate of 2 - 3.5 L air/L sample/min, ammonia removal was 95% from limed leachate (pH 11.5) which contained 137 - 330 mg/L NH_x-N (Diamadopoulos, 1994). Liming also indirectly removes portion of COD, color, phosphorus and metals by precipitation and coagulation. Air stripping removed about 70% ammoniacal-nitrogen from landfill leachate; liming to pH > 10 raised the removal efficiency to 82 - 93% (Cheung *et al.*, in press).

1.5.1.5 Reverse osmosis (RO)

The process consists of passing a liquid through a semi-permeable membrane (such as cellulose acetate and nylon) at pressure up to 10,000 kN/m^2. The membrane rejects most of the ions and molecules while permitting water passages at an acceptable rate. RO is especially useful as a post-biological treatment option which mainly removes low molecular weight organic compounds, colloidal particles and suspended solids (Jans, 1992; Weber and Holz, 1992).

Single stage RO process could achieve over 80% removal with respect to COD and BOD. However, a second stage is recommended for higher removal efficiency for ammonia and chloride (Weber and Holz, 1992).

Small molecules, such as ammonia and low molecular weight organics, are not effectively removed. Regular maintenance is needed for RO plants treating leachate due to the high concentration of organics and the precipitation of inorganics.

Fouling, biofouling and sealing of membrane are common. Energy consumption is very high as high pressure is required. The most serious problem is the production of concentrated wastes. This concentrated wastes are usually disposed of in landfill, but this practice is no longer acceptable as the concentrate is now regarded as a hazardous waste which requires special disposal. Expensive processes such as evaporation and drying are required to treat the concentrated residue prior to disposal.

1.5.2 Biological treatment

Both aerobic and anaerobic processes have been studied widely. However, in a long term basis, aerobic treatment is more economic and flexible in operation, because the organic strength of landfill leachate decreases with the stabilization (age) of landfill, whereas anaerobic process is effective for high organic strength leachate only. Anaerobic reaction is more sensitive to variable loads, organic shock and toxic compounds. High strength acetogenic leachate may contain high concentration of heavy metals which affect the anaerobic microorganisms in the digestion tank. Furthermore, anaerobic treatment for landfill leachate cannot remove ammonia. Therefore, it becomes uneconomic to construct an anaerobic treatment plant which is higher in capital cost but with efficiency declining rapidly.

1.5.2.1 Aerobic treatment

Aerobic systems widely examined for leachate treatment include suspended growth system such as activated sludge process, sequencing batch reactor, aeration lagoon, as well as attached growth system such as trickling filter and rotating biological contactor. Most of the techniques are designed for municipal wastewater

treatment, and cannot be adopted completely for leachate treatment because strength and composition of leachate are so different from that of sewage, e.g. much higher ammonia concentration, presence of toxic compounds and high concentration of refractory carbon.

Leachate is usually low in phosphorus and cannot support a good growth of biomass. Phosphate, such as sodium dihydrogen phosphate or phosphoric acid, is added to achieve a BOD:P ratio of 100:1. Too low a phosphorus concentration would significantly affect effluent quality. When the BOD:P ratio was elevated from 100:1.1 to 100:0.3 and 100:0.1, effluent BOD increased from 55 to 300 and 1430 mg/L respectively, total nitrogen from 15.1 to 109.2 and 149.3 mg/L respectively and suspended solids from 133 to 245 and 1805 mg/L respectively (Mavinic, 1986). However, in actual conditions, organic carbon level varies from time to time, and extensive monitoring is required in order to keep the BOD:P ratio at optimum. Addition of phosphate also increases the cost of leachate treatment and may raise its concentration in the final effluent.

pH is another key parameter that has to be controlled in aerobic treatment. Leachate usually has a high ammonia concentration, and nitrification may occur at the same time of carbonaceous removal. If nitrification occurs, alkalinity in the leachate may not be enough to buffer the H^+ formed during nitrification. Alkali is usually added to prevent the acidification of the aerated leachate.

1.5.2.1.1 Activated sludge system

Activated sludge system is studied most extensively for leachate treatment (Bull *et al.*, 1983, Chian and DeWalle, 1976; Keenan *et al.*, 1984; Knox, 1983 and

1985; Robinson and Maris, 1983 and 1985). Although variation in leachate composition prevails optimal operational conditions of leachate treatment by activated sludge process have been generalized (Qasim and Chiang, 1994). In order to obtain 90 - 99% carbon removal, mixed liquor volatile suspended solid (MLVSS) concentration at 5,000-10,000 mg/L, food to microorganisms ratio (F/M) at 0.02 - 0.06 d^{-1}, hydraulic retention time (HRT) at 1 - 10 days, and sludge retention time (SRT) at 15 - 60 days should be maintained. Activated sludge system showed a better performance in treating young leachate than old leachate (Robinson and Maris, 1985). COD and BOD removal efficiencies of young leachate were > 97% and > 99% respectively when the sludge retention time (SRT) was 20 days or higher. About 60% of ammonia was converted to biomass and 38% was oxidized to nitrate. However, when old leachate was treated, COD removal level dropped to 41 - 43% under the same operational conditions due to the higher proportion of refractory carbon in the old leachate. Increase of HRT could not improve treatment efficiency as carbon removal would stop after degradable carbon was removed (Bull *et al.*, 1983).

High concentration of ammonia in landfill leachate causes another problem in activated sludge system. When metabolic uptake by heterotrophic bacteria was employed as major route of ammonia removal, complete ammonia removal was only possible if C:N ratio did not exceed 100:3.6 (Robinson and Maris, 1983). Moreover, the NH_3-N concentration must be lower than 200 mg/L, otherwise sludge settleability would be seriously affected. Sludge volume increased with the ammonia concentration even though settleability could be maintained at reasonable level. Sludge volume increased from 10.4% to 48.8% of the reactor volume when influent ammonia concentration increased from 80 to 407 mg/L N.

Operational problems reported in leachate treatment by activated sludge process include foaming, precipitation of iron and carbonates, excessive sludge production and decrease in efficiency during winter. Conventional activated sludge method is less appreciated at landfill as it demands greater operational skill.

1.5.2.1.2 Aeration lagoon

Aeration lagoon provides the least expensive and simple option for leachate treatment. Completed landfill site also provides a large area for the construction of aeration lagoon. Long HRT of leachate allows the development of biomass to replenish those removed and no sludge recycling is required. The demand for operation skill and maintenance is low.

Aeration lagoon appears to be promising in treating high organic strength leachate. Field-scale aerated lagoon operated in the Bryn Pesteg Landfill (United Kingdom) since 1983 for treating high strength leachate (COD of 9750 mg/L, BOD of 7000 mg/L and NH_x-N of 175 mg/L) had a very high efficiency even at winter time when the lagoon temperature was only 4°C (Robinson, 1992). Leachate with COD of 4140 - 13250 mg/L and BOD of 1630 - 11713 mg/L could also be treated efficiently by batch-scale aeration lagoon (Young *et al.*, 1987). Removal of BOD and COD were 90% and 99% respectively at HRT of 3 - 21 days. However, efficiency of ammonia removal varied from 54.9 to 98% for influent with 87 - 348 mg/L NH_x-N.

There were also successful experience in treating leachate with medium strength by aeration lagoon. In the Whiteriver Landfill (United Kingdom), aeration lagoon for leachate with COD of 1733 mg/L, BOD of 980 mg/L and NH_x-N of 104 mg/L produced effluent with COD of 291 mg/L, BOD of 39 mg/L and NH_x-N of 0.19

mg/L (Robinson, 1992). Although BOD:N ratio was much lower than 100:5 (only 47.1:5), treatment process was not affected and both BOD and ammonia were reduced to very low levels. In the Compton Basset Landfill (United Kingdom), a methanogenic leachate with COD of 1500 mg/L, BOD of 400 mg/L and NH_x-N of 600 mg/L was treated by an aeration lagoon (Robinson, 1987 and 1990). Effluent from a jam factory was added into the lagoon to increase the carbon level of the mixed liquor so as to provide adequate carbon for the growth of heterotrophic bacteria. At a HRT of 25 days, effluent with COD of 268 mg/L, BOD of 10.4 mg/L and NH_x-N of 3.4 mg/L was produced. Although ammonia was reduced to a very low level, metabolic uptake by biomass only accounted for 15% of removal; 33% of ammonia was oxidized to nitrate via nitrification. It is believed that stripping and the combined action of nitrification and denitrification may account for the other 50% nitrogen loss.

1.5.2.1.3 Sequencing batch reactor

The sequencing batch reactor (SBR) utilizes the same tank to aerate, settle and recycle solids and is seen as a good option with low flow application. SBR is less likely to be damaged by scaling of calcium and magnesium ions in leachate. Maintenance is simple and easy. Another advantage is the high flexibility of the treatment process in terms of the length of aerobic/anaerobic/settling period. Operation schedule can be adjusted to comply with the change of leachate composition. Compared with activated sludge process and aeration lagoon, SBR has attracted less attention in landfill leachate treatment. SBR has been used to treat leachate with 100 - 330 mg/L NH_4-N, 27 mg/L BOD and 100 - 150 mg/L COD (Hosomi *et al.*, 1989). Ammonia removal greater than 90% was achieved at loading

rate less than 0.05 kg/m^3/d NH_4-H and both aerobic and anoxic periods were longer than 4 hours. COD removal ranged from 37.6% to 50.8%.

1.5.2.1.4 Trickling filter

A pilot-scale trickling filter has been used to treat landfill leachate with low BOD (32 - 291 mg/L) and high ammonia concentration (140 - 520 mg/L) (Knox, 1985). Effluent produced had BOD of 8 - 40 mg/L and NH_x-N smaller than 1.0 - 34 mg/L. Trickling filters generally had better performance than the activated sludge tested in parallel but required lower hydraulic loading rate. Trickling filters are less susceptible to low temperature than activated sludge system.

However, trickling filters are not appropriate for treating high-strength leachate as the filter will be clogged by excessive biofilm growth and organic deposition on the filter medium (Britz, 1995). They are also susceptible to blocking due to the inorganic precipitation (such as iron carbonate and manganese carbonate) which results in the reduction of microbial activity.

1.5.2.1.5 Rotating biological contactor (RBC)

In leachate treatment, RBC was studied more widely than trickling filter. RBC with a specific surface area of 180m^2/m^3, rotation rate of 1.5 rpm and loading rate of 1.6 - 5.4g N/m^3/d was used to treat a low strength leachate with BOD less than 30 mg/L and ammonia concentration about 100 - 340 mg/L (Knox, 1992). Effluent produced had an average BOD of 14 mg/L and NH_3-N concentration of 2.96 mg/L. Performance was dependent on temperature with concentration of ammonia exceeding 10 mg/L N occasionally when heating system failed. Suspended solids

were not removed efficiently as nitrification was the major biological process involved, with little flocs formed to trap the suspended solids.

Higher strength leachate with COD of 254 mg/L, BOD of 5340 mg/L and NH_x-N of 196 mg/L has been successfully treated by a 4-stage RBC with specific surface area of 150 m^2/m^3, rotation rate of 4.6 rpm and loading rate of 1500 g $COD/m^3/d$ (Laughlin *et al.*, 1992). The average COD and BOD removal efficiencies were 86% and 95% respectively. RBC also showed a very good biosorption ability. Concentrations of metals, such as iron, manganese, zinc and aluminum, were reduced by 82 - 93% after treatment.

Modified RBC system enhances the removal of nitrogen by nitrification and denitrification (Hosmi *et al.*, 1991; Spengel and Dzmbak, 1991). A 3-stage aerobic and 1-stage anaerobic RBC with rotation speed of 2.3 rpm and loading rate of 2.8 - 18.5 g COD/m^2 (equivalent to 1.2 - 7.3 g NH_3-$N/m^2/d$) was used to treat leachate containing 15 - 38 mg/L BOD, 322 - 358 mg/L COD and 148 - 160 mg/L NH_3-N (Spengel and Dzmbak, 1991). Most of the BOD was removed while only 30 - 38% of COD was removed in the aerobic stage. Efficiency decreased with increasing hydraulic loading. The average effluent of aerobic stage contained less than 5 mg/L N while nitrate concentration was increased by approximately the same amount of ammonia oxidized. The ammonia removal efficiency, like COD removal, decreased with increasing hydraulic loading. In the anaerobic stage, methanol was added at a rate of 1.75 mg/mg NO_3-N/L. Nitrate concentration was reduced from 130 to 75 mg/L N Aerobic-anaerobic RBC was superior to standard RBC in terms of COD and nitrogen removal (Hosmoi *et al.*, 1991).

1.5.2.2 Anaerobic treatment

Although anaerobic process can effectively remove organic carbon of young landfill leachate with high BOD:COD ratio (Qasim and Chiang, 1994), the efficiency in nitrogen removal is low as only very small amount of nitrogen could be removed in the form of nitrogen gas during anaerobic process (Cameron and Koch, 1980). At HRT of 5 to 20 days, only 2.3 - 13.1% of total nitrogen was removed. In terms of carbon removal, performance of anaerobic processes was also poor than aerobic one (Gourdon *et al.*, 1992). TOC removal of aerobic and anaerobic processes was 70.3% and 35.9% respectively. Gel permeation chromatography identified that the same group of molecules was resistant to aerobic and anaerobic degradation. However, these molecules were almost non-degradable (or were degraded at a very low rate) under anaerobic conditions whereas they were biodegradable to about 50% in aerobic conditions.

Nevertheless, anaerobic processes still receive considerable attention because they offer several benefits over aerobic processes. In anaerobic reactor, part of the carbon is converted to methane and carbon dioxide, which results in lower production rate of biological solids. Methane produced by anaerobic process can be used as a source of energy for maintaining high operation temperature (usually above 30°C). Anaerobic systems are generally seen as more economical as they do not have the high energy requirements associated with aeration in aerobic systems. Hydrogen sulfide produced by anaerobic process also precipitates most heavy metals; anaerobic reactor is good at removing metals in leachate (Cameron and Koch, 1980; Kennedy *et al.*, 1988).

Different anaerobic systems were studied for leachate treatment, including

conventional digester, downflow and upflow anaerobic filters, upflow anaerobic sludge blanket (UASB) reactor and hybrid reactor of anaerobic filter and UASB reactor.

In conventional digestion process, microorganisms and substrate (leachate) are kept in contact by means of mixing, with occasional sludge recycle. COD removal of 90 - 96% could be obtained at loading of 0.43 - 1.22 kg COD/m^3/d at HRT of 5 to 20 days and temperature of 23 to 30°C (Boyle and Ham, 1974). Similar efficiency was obtained in another study under similar operational conditions (Cameron and Koch, 1980). COD removal of 84 - 90% was observed at loading of 0.26 to 1.08 kg COD/m^3/d at HRT of 5 to 20 days and temperature of 34°C. Increase of SRT increased carbon removal efficiency but decreased the percentage yield of methane in the biogas produced.

Anaerobic filters can retain more microorganisms than conventional completely-mixed digesters. Flow of wastewater in the filter may either be upward (upflow anaerobic filter) or downward (downflow anaerobic filter). An upflow anaerobic filter has been used to treat a strong leachate with COD of 14000 mg/L and BOD:COD ratio of 0.7 and a medium strength leachate with COD of 3750 mg/L and BOD:COD ratio of 0.3 (Henry *et al.*, 1987). The anaerobic filter could reduce the COD of both leachates by 90% at loading ratio of 1.26 - 1.45 kg COD/m^3/d. No phosphorus addition was required even the COD:P ratio was as high as 17900 - 30640. Anaerobic process has much lower phosphorus requirement than aerobic process which requires a C:P ratio of 100:1. A downflow anaerobic filter with HRT of 5.8 days and organic loading rate of 1.5 kg COD/m^3/d had COD and BOD removal efficiency of 72% and 80% (Laughlin *et al.*, 1992).

Upflow anaerobic sludge blanket (UASB) reactor is another commonly employed anaerobic system for leachate treatment. UASB has wastewater flows upwards through a dense bed of active granular sludge with good settling properties, which then flows through a less dense blanket of suspended flocs. Effluent leaves the reactor via a gas-solid separation device at the top of reactor. When treating a high strength leachate with COD of 11,450 - 33,440 mg/L, BOD of 886 - 9,320 mg/L, UASB reactor achieved over 80% carbon removal at loading rate of 3.6 - 19.7 kg COD/m^3/d at 28 - 32°C (Blakey *et al.*, 1992). In some cases, UASB reactor was combined with anaerobic filter to enhance solid retention. Hybrid reactor had better performance than downflow anaerobic filter in treating a lime - treated leachate with COD of 19,480 mg/L (Kennedy *et al.*, 1988). Reactor used in this study had a packing media at upper part that acted as anaerobic filter. Lower portion operated as an UASB reactor. Increase of organic loading rate from 5.2 to 14.7 kg COD/m^3/d resulted in decrease of COD removal from 97 to 94% in downflow anaerobic filter whereas COD removal of hybrid reactor remained at 97% even when loading rate increased from 4.8 to 14.9 kg COD/m^3/d. A similarly designed reactor removed 85 to 90% COD at organic loading rate of 9 kg COD/m^3/d and 35°C (Keenen *et al.*, 1992).

Both anaerobic filter and hybrid reactor of UASB and anaerobic filter are likely to suffer from clogging during leachate treatment. Large amount of inorganic precipitates accumulated within the reactor and piping after a certain period of operation (Keenan *et al.*, 1992; Kennedy *et al.*, 1988). Carbonates of iron, calcium and manganese were responsible for up to 80% of the fixed solids in the reactor. In UASB reactor, when inorganic precipitates are allowed to accumulate on sludge surface, organisms inside undergo endogenous metabolism and gases produced are

trapped within the flocs which results in the formation of floating sludge.

For anaerobic treatment process, high temperature must be used in order to maintain high efficiency. When organic loading of upflow anaerobic filter was increased from 0.29 to 1.10 kg COD/m^3/d, efficiency of reactor declined rapidly from 46 to 20% at 20°C while efficiency remained at 44 - 51% at 37°C (Mendez *et al.*, 1989).

1.5.3 Co-treatment with municipal wastewater

If a municipal wastewater treatment plant is located in the vicinity of landfills and has enough capacity to receive additional flow, leachate can be piped to the plant for combined treatment with domestic sewage. In general, leachate flow must be maintained at lower than 5% of the total input and leachate must consume less than 10 g O_2/L (Britz, 1995; Harrington and Maris, 1986). Addition of leachate would not affect the removal efficiency for organic carbon and ammonia if operational conditions are adjusted properly (Ahnert and Ehrig, 1992). With the addition of leachate, oxygen consumption and sludge volume are increased, oxygen supply and sludge handling processes must be increased accordingly (Kelly, 1987). Other problems induced by co-treating leachate with sewage include the decrease of floc settleability, higher effluent ammonia concentration, precipitation of iron oxides, scum formation and corrosion of piping system.

Co-treatment of leachate with municipal sewage is not always an appropriate option. Nitrogen in the sewage stream is already a problem to sewage treatment plant. Special steps must be adopted to remove excess nitrogen (Wu, 1994). The delivery of nitrogen-rich leachate to sewage works will further increase the nitrogen

load. Moreover, landfill sites may not be located near sewage treatment plant. Construction of piping system for leachate is necessary if co-treatment is employed.

1.5.4 Recirculation

When leachate is returned back to landfill, landfill itself can act as an anaerobic trickling filter. Physical and biological (mainly anaerobic) reactions facilitate the removal of pollutants and result in the formation of a lower strength leachate. Recirculation increases moisture content of the wastes, and promotes degradation and stabilization (Tittlebaum, 1982). Portion of leachate evaporates when it is sprayed onto the surface of landfill, which reduces the total volume of leachate.

The hydrology of the site should be monitored carefully and precaution must be taken to prevent the formation of channels and lateral movement into surrounding surface or ground water. The final soil cover should be furrowed regularly in order to increase infiltration of leachate (Barrer and Maris, 1992). Prolonged recirculation increases the chance of lateral seepage of leachate. Recirculation may not be possible if the water balance indicates an accumulation of liquid in the fill.

Recirculation is ineffective with respect to the inorganic fraction in leachate. Although over 90% COD and 98% BOD were removed after the leachate was recirculated to a closed region of landfill, NH_x-N concentration increased by 78.5% due to the degradation of nitrogenous compounds in the buried wastes (Diamadopoulos, 1994). Recirculation alone cannot provide a final solution for leachate treatment. A further treatment step must be considered when direct discharge to the environment is required (Barrer and Maris, 1992; Young *et al.*,

1987).

1.5.5 Irrigation

Irrigation is a land application system employing soil and plants for treatment. Soil can filter suspended solids and can remove dissolved components by adsorption, ion exchange and precipitation. Plants utilize nutrients in leachate for growth, and evapotranspiration reduces leachate volume. When irrigation rate was consistent with evapotranspiration demands, leachate enhanced the growth of red maple (*Acer rubrum*) and sugar maple (*Acer saccharum*) seedlings (Gordon *et al.*, 1989). Irrigating orchard grass (*Dactylis glomerata*) and willow (*Salix viminalis* and *S. dasyclados*) with a low strength leachate (COD of 190 mg/L, BOD of 17 mg/L and NH_x-N of 84 mg/L) increased the biomass production and reduced leachate volume through evapotranspiration (Hasselgren, 1992). Orchard grass and willow consumed water equivalent to 500 - 700 mm and 1,200 - 1,500 mm water per annum through evapotranspiration. More than 95% ammonia was removed by assimilation by plants and oxidation by soil microorganisms. Another UK practical experience showed that at a loading rate of $45m^3$/ha/d, BOD was reduced from 300 to 15 mg/L and NH_x-N from 50 to 5 mg/L when leachate was treated by spray irrigation (Harrington and Maris, 1986).

However, irrigation is not a popular treatment option. Irrigation is only suitable for low strength leachate; otherwise, problems of sanitation, odor and pest must be considered. Toxicity of ammonia and other compounds to plant is another problem. Prolonged irrigation may induce damage of soil structure and phytotoxic compounds (e.g. heavy metals and trace organics) may accumulate in the soil. Large

area is required to receive a substantial volume of leachate. Irrigation is not a suitable option for leachate treatment in places as Hong Kong where land is scarce and most of the old landfills are located near urban area.

1.6 Aims of the Thesis

Landfilling is the sole method for solid waste disposal in Hong Kong. Unfortunately, many of the early landfills were inadequately designed, constructed and operated, and were below the present environmental standards. Unless proper measures are taken in time, our surrounding environment will deteriorate. Because of the complexity of the wastes disposed of in landfills and the difference in the stages of decomposition, leachates from different landfills can vary very much in quality. Knowledge of the physico-chemical properties of leachate not only provides information of its treatability but also its possible effects to our environment and organisms in the receiving water. In Chapter 2, the quality of leachates from Ma Yau Tong Central Landfill, a closed site, and Pillar Point Valley Landfill, an operating site, were monitored for twelve months. The results could be used to provide information on choosing the suitable method for treating leachates from landfills of different age. The correlation of leachate composition to rainfall was also studied to evaluate the possibility of predicting leachate quality by rainfall data.

There is a complex variety of compounds present in a leachate and many of them are hazardous to the environment. Determination of every possible potentially hazardous chemical is impossible. Biological toxicity test is therefore used as a complementary tool to chemical analyses to determine the ecotoxicological effects of a leachate. In Chapter 3, leachates collected from both landfills in wet and dry

months were assessed by bacterium (Microtox test), alga (*Chlorella pyrenoidosa*), water flea (*Moina macrocopa*) and zebrafish (*Brachydanio rerio*). The suitability and sensitivity of these four organisms for testing landfill leachate were compared. Concentrations of some toxic compounds such as phenols, heavy metals and cyanide in leachate were also determined.

According to the results of Chapter 2, leachates from Ma Yau Tong Central and Pillar Point Valley Landfills were high in ammoniacal-N (more than 500 mg/L) but low in biodegradable carbon (usually below 100 mg/L). Most of the previous research on leachate treatment have focused on carbon removal. Ammonia removal of leachate treatment is not studied extensively even though ammonia is a major pollutant in leachate on a long term basis. Therefore the treatability of leachate by nitrification and denitrification was studied. Efficiency of nitrification in removing ammonia in leachate under different phosphorus levels, hydraulic retention time and organic carbon dosages was studied using a laboratory scale continuous flow system. The efficiency of system was assessed through monitoring ammonia removal, as well as nitrite and nitrate formation. Populations of nitrifiers and heterotrophic bacteria, major microorganisms for the removal of ammonia and carbon respectively, were determined (Chapter 4). In Chapter 5, denitrification of the nitrified leachate under different hydraulic retention time was also studied by another laboratory scale continuous flow system.

It is hoped that a thorough understanding of leachate properties in terms of its chemical quality and ecotoxicity can help in assessing its effects on the environment, and, more importantly, designing an appropriate and efficient purification system which can serve a municipal landfill throughout its operational life-span.

2 CHARACTERIZATION OF LANDFILL LEACHATE

2.1 INTRODUCTION

In Hong Kong, there was little concern on leachate problem in the past. Leachate was allowed to accumulate in various points in the landfills. For those landfills adjacent to coastal area (e.g. Shuen Wan Landfill and Pillar Point Landfill), leachate was finally poured into the coastal water without treatment (Waste Disposal Authority, 1989). Due to tighter environmental control, leachate needs to be treated prior to discharge. A comprehensive landfill restoration study was initiated in 1990 to restore all the completed urban landfills. Restoration works, including the construction of leachate treatment plant, had begun in 7 different landfills of the territory (Environmental Protection Department, 1993).

The design of a leachate treatment system requires extensive characterization of leachate and accurate prediction of the fluctuation in leachate properties in the future when the fill is stabilizing. Underestimating the strength of leachate will result in the production of poor quality effluent that cannot meet the discharge standards. Overestimation of leachate strength causes the uneconomic use of treatment plant and this is not uncommon. In GROWS Landfill of Pennsylvania, treatment plant designed for 550 m^3/d only received about 200 m^3/d (Knox, 1985). In a landfill in Dorest, England, treatment plant was designed for influent with 19000 mg/L BOD and 250 mg/L NH_3. However, influent received had BOD of about 150 mg/L and NH_3 level about 75 mg/L (Porter, 1981). In Pitsea Landfill, England, a rotating biological contactor (RBC) plant which was designed for treating 50 - 300 mg/L BOD and 80 - 600 mg/L NH_3-N only received leachate with about one-half of ammonia level and BOD rarely exceeding 30 mg/L (Knox, 1992).

Age of a landfill affects leachate quality. Leachate produced from stabilized landfills such as Gin Drinkers Bay (Chu *et al.*, 1994), Ma Yau Tong and Shiu Lang Shui (Carville and Robinson, 1991) is in general low in organic strength. Variation in quantity affects leachate quality to a greater extent than the degradation process of the landfill. Recently closed or operating landfills produce leachate the quality of which depends on quantity of leachate and the degree of degradation. This category of landfills includes Jordan Valley, Pillar Point Valley, Shuen Wan, Tseung Kwan O and the three new strategic landfills. Variation of leachate quality due to stabilization processes must be considered in the design of leachate treatment scheme to allow modification of the treatment process when significant change of leachate quality occurs.

Analyses of the major chemical parameters, such as COD, BOD and nitrogen can provide information on the process of biological decomposition within a landfill. They also indicate the major pollutants that must be removed if leachate is to be discharged and may suggest whether a biological, physicochemical, or some combined treatment processes are likely to be the most efficient. Temporal variation study also provides information in designing a treatment system for the leachate.

Biological activities in landfill are responsible for the stabilization of landfill and the composition of leachate (Section 1.2). The biological and chemical composition of leachate determine the treatability of leachate by biological processes. However, contrary to the chemical characteristics of leachate, data on biological composition are scarce.

The main objectives of the present experiment were (1) to study the chemical properties of leachate from a closed landfill and an operating landfill, (2) to identify the major pollutants in leachates from the two landfills of different age, (3) to study

the temporal variation in leachate quality and the correlation between rainfall and leachate composition, (4) to understand the biological activities of landfill leachate and (5) to generate background information for treatment plant design.

2.2 Materials and Methods

2.2.1 Description of landfill sites

Ma Yau Tong Central (MYT) Landfill and Pillar Point Valley (PPV) Landfill, were chosen for the study. Both are valley fills. MYT Landfill is located in eastern Kowloon. It started to operate in 1980 and was closed in 1986. It occupies 9.9 hectares and had received a million tonnes of wastes.

PPV Landfill, located in western New Territories, started to receive wastes in 1983 and is still under operation. It occupies a total area of 34 hectares and has already been filled with 13 million tonnes of solid wastes (Environmental Protection Department, 1995). It also serves as a co-disposal site for some chemical wastes that are not allowed to treat in the Chemical Waste Treatment Facilities at Tsing Yi. These include tannery offcuts, industrial wastewater treatment sludge and the residue of the Chemical Waste Treatment Plant. As the landfill has been operated for more than 11 years at the time of sampling, leachate collected from the site would be a mixture of different decomposition phases leachates. Study of the PPV Landfill leachate may provide some hints on the quality of leachate collected from the new strategic landfills. Due to the high temperature and rainfall of Hong Kong when compared with other western counties, landfills in Hong Kong are stabilized at much faster rate (Carville and Robinson, 1991). Leachate from the strategic landfills would soon have properties similar to the PPV leachate.

2.2.2 Leachate collection

Leachates were collected from Ma Yau Tong Central (MYT) Landfill and Pillar Point Valley (PPV) Landfill every two months from April 1994 to February 1995. The collected leachate was stored in 4.5 L plastic carboys. Samples were kept at 4°C upon arrival to laboratory. Samples that could not be analyzed within 24 hours after collection were preserved and stored in ways as recommended by 'standard methods' (American Public Health Association, 1992) within six hours of sampling.

2.2.3 Chemical analysis

pH, dissolved oxygen (DO), electrical conductivity (EC) and salinity were determined immediately upon arrival to laboratory. The leachate was analyzed for pH by a pH electrode connected to an Orion 902A analyzer, DO by a YSL dissolved oxygen meter, EC by a Corning Checkmate conductivity meter, and salinity by an Atago hand refractometer. Samples for the determination of biochemical oxygen demand (BOD) and total solids (TS) were kept at 4°C and analyzed within eight hours of sampling according to 'standard methods' (American Public Health Association, 1992).

Samples for the determination of chemical oxygen demand (COD), total Kjeldahl nitrogen (TKN) and total Kjeldahl phosphorus (TKP) were acidified with sulfuric acid to pH 2 and stored at 4°C before determination. COD was determined by open reflux method (American Public Health Association, 1992), TKN by semi-micro Kjeldahl digestion followed by salicylate nitroprusside method using a Lachat QuickChem AE Automated Ion Analyzer, and TKP by semi-micro Kjeldahl digestion followed by ascorbic acid method using the Automated Ion Analyzer.

Samples for ammoniacal-nitrogen (NH_x-N) and oxidized-nitrogen (NO_x-N) were filtered through Advantec 5C filter paper, then acidified with sulfuric acid to pH 2 and stored at 4°C before measurement. NH_x-N and NO_x-N were determined by phenate method and brucine method respectively. Samples for orthophosphate-phosphorus (PO_4^{3-}-P) were filtered through Advantec 5C filter paper, then acidified with sulfuric acid and frozen before determination by ascorbic acid method.

For the determination of total and soluble metals, raw and filtered (by Advantec 5C filter paper) leachates were acidified with nitric acid to pH 2 and stored at 4°C before further treatment. Metal concentrations were measured by nitric acid digestion followed by determination using a Hitachi Z8100 Polarized Zeeman atomic absorption spectrophotometer.

Samples for carbohydrate analysis were acidified with sulfuric acid to pH 2 and kept frozen before determination by anthrone method (Raunkijær *et al.*, 1994). Samples for measurement of protein were preserved by adding of sodium dodecyl sulfate to 1%. Samples were kept at 4°C before determination of protein concentration by Lowry method (Raunkijær *et al.*, 1994). Samples for measurement of lipids were acidified with hydrochloric acid to pH 2 and kept frozen before determination by partition-gravimetric method (American Public Health Association, 1992).

2.2.4 Biological analysis

Microbial population was determined for the samples collected in August 1994 and February 1995 from both MYT and PPV Landfills. The collected leachate was kept in a sterile 250 mL glass bottle at 4°C and analyzed within six hours after collection.

Populations of total heterotrophic bacteria, carbohydrate-utilizing bacteria, protein-utilizing bacteria and lipid-utilizing bacteria were determined by spread plate method on plate count agar (American Public Health Association, 1992), starch agar (Ronald, 1993), milk agar (Ronald, 1993) and tributyrin agar (Ronald, 1993) respectively. Formula and preparation of the selective media are shown in the Appendix 1. The plates were incubated at 35±1°C for 2 days before plate observation and colony counting. Population of fungi was determined by spread plate method on neopeptone-glucose-rose Bengal aureomycin agar (American Public Health Association, 1992). Formula are shown in the Appendix 1. The plates were incubated at 22±2°C for 5 - 7 days.

2.2.5 Statistical analysis

Two-way Analysis of Variance (ANOVA) of untransformed data was carried out to test the difference of leachate quality among different months and between the two landfills. Tukey's Honestly Significant Difference Test was employed when difference was detected by two-way ANOVA. Correlation between leachate quality and cumulative rainfall was studied by Pearson Correlation. All statistical analyses were performed by means of SPSS (Statistical Package for Social Science) for Windows Release 6.0 of SPSS Inc.

2.3 Results and Discussion

2.3.1 Chemical properties of leachate

According to two-way ANOVA test, differences of leachate quality were found between the two sites and among the six different sampling times. Least significant differences (LSD) by Tukey's Honestly Significant Difference Test were calculated and are shown in Figs. 2.1 - 2.8.

Both sites had neutral leachate, with pH of the MYT leachate slightly higher than that of the PPV leachate (Table 2.1). The neutral pH was partly due to the removal of fatty acids by methanogenic bacteria (Andreottola and Cannas, 1992). Methanogenic stage (Phase III) was likely to be reached within a very short period of time under wet and hot conditions (Carville and Robinson, 1991) prevail in Hong Kong. MYT Landfill was closed since 1986. PPV Landfill started to receive wastes at 1983 and is still an operating site. Age of deposited wastes ranged from one day to 11 years. The leachate collected was a mixture of leachates from wastes of different degree of decomposition, the resultant pH of which depended on the relative contribution of leachates of different ages. The methanogenic leachate produced may have neutralized the acidic acetogenic leachate. Chemical analysis of the June 1994 and December 1994 samples reveals that concentration of carboxylic acids from the older MYT leachate (16.3 and 34.4 mg/L) was lower than those of the PPV leachate (114 and 127 mg/L) (Table 3.1). This agrees with the age of landfill.

Both leachates had relatively low DO and all PPV samples had lower DO than MYT samples (Fig. 2.1). The higher organic content of PPV leachate which resulted in higher oxygen demand might account for the lower DO. Other factors, such as the flow of leachate before reaching the sampling point, would also affect the DO of

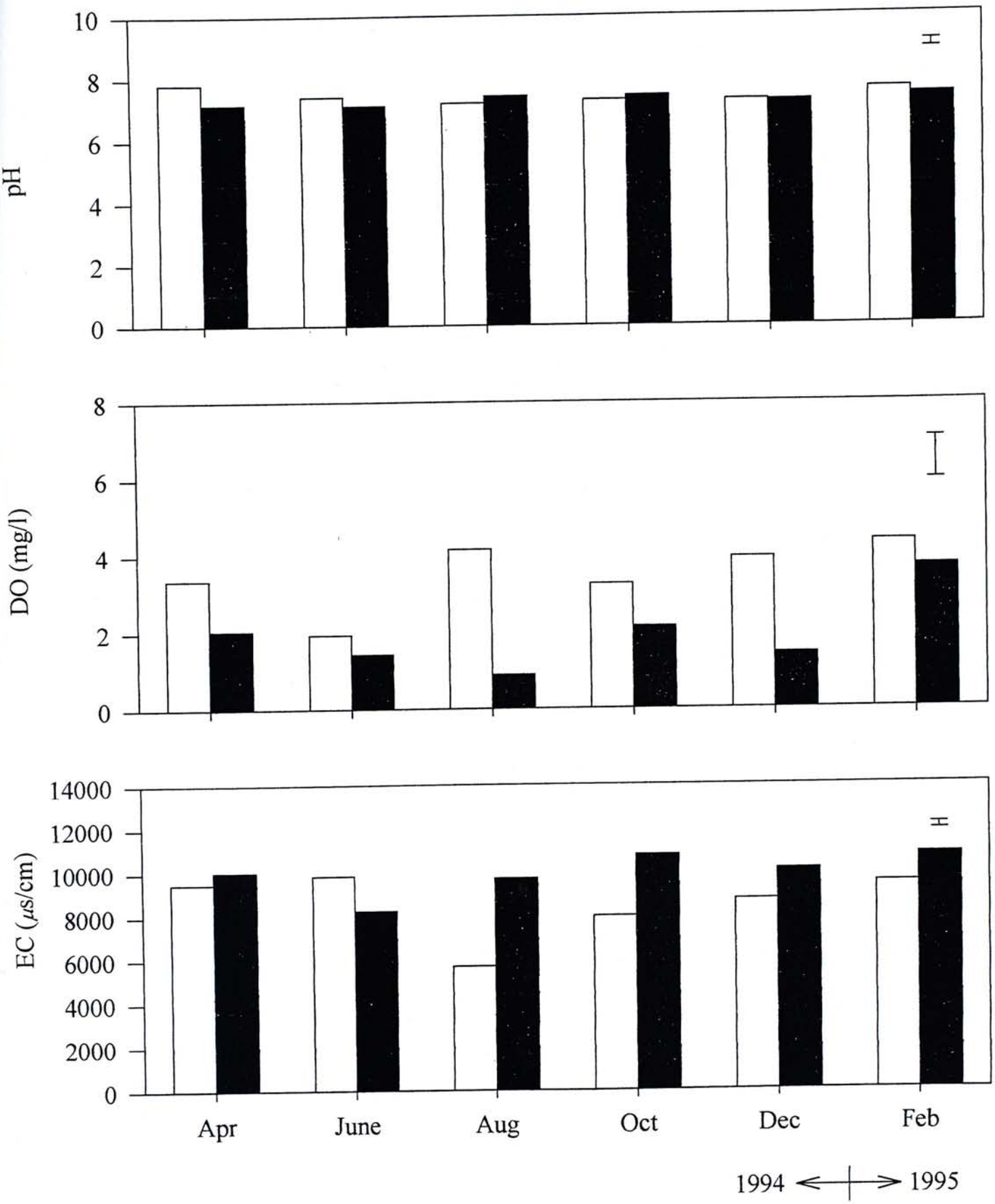


Fig. 2.1 pH, dissolved oxygen (DO) and electrical conductivity (EC) of leachates collected from MYT Landfill (empty bar) and PPV Landfill (solid bar) from April 1994 to February 1995. Values shown are the means of 4 replicates. Vertical bars denote LSD by the Tukey's Honestly Significant Difference Test at $P = 0.05$.

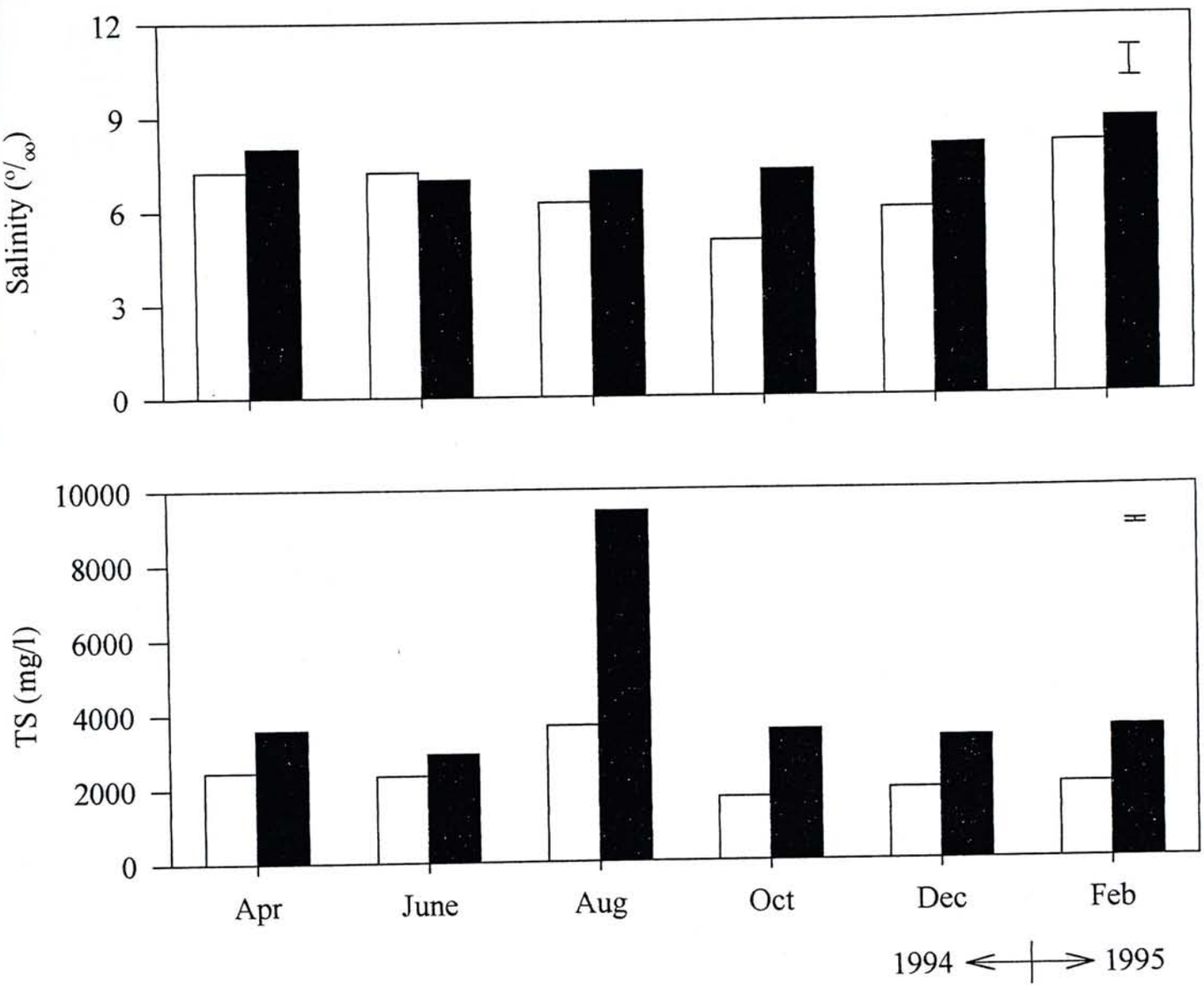


Fig. 2.2 Salinity and total solids (TS) of leachates collected from MYT Landfill (empty bar) and PPV Landfill (solid bar) from April 1994 to February 1995. Values shown are the means of 4 replicates. Vertical bars denote LSD by the Tukey's Honestly Significant Difference Test at $P = 0.05$.

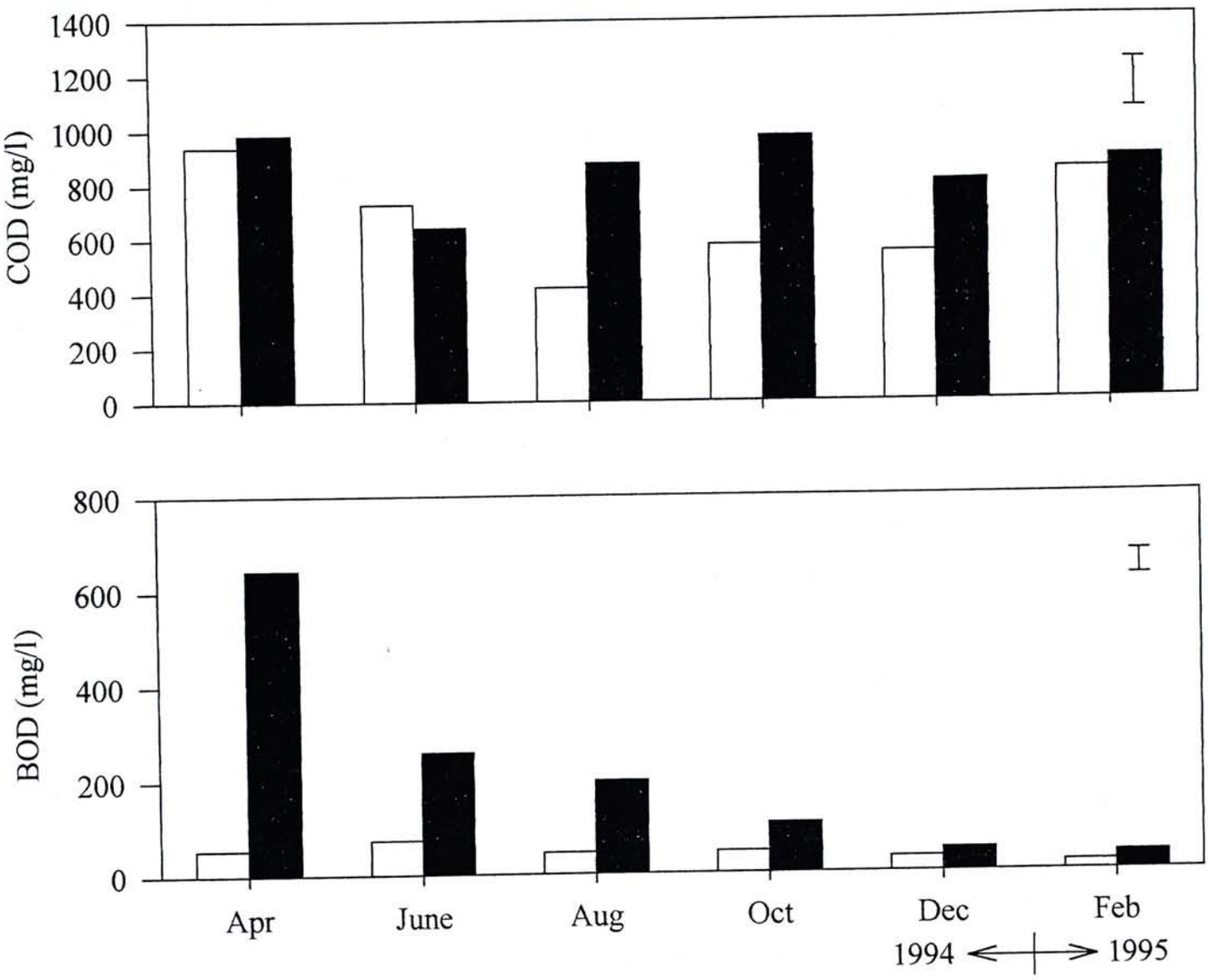


Fig. 2.3 COD and BOD of leachates collected from MYT Landfill (empty bar) and PPV Landfill (solid bar) from April 1994 to February 1995. Values shown are the means of 4 replicates. Vertical bars denote LSD by the Tukey's Honestly Significant Difference Test at $P = 0.05$.

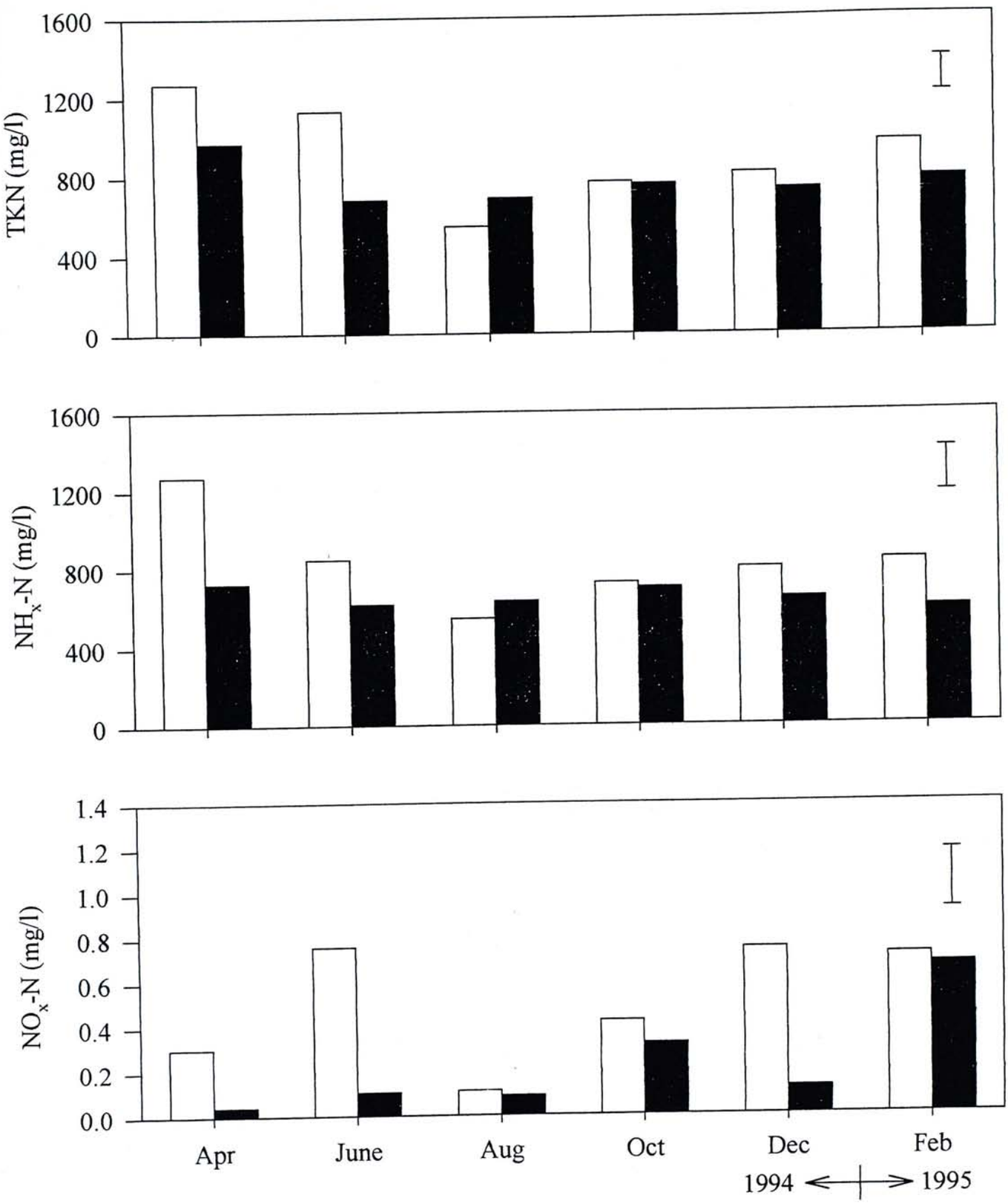


Fig. 2.4 Concentrations of total Kjeldahl nitrogen (TKN), ammoniacal-nitrogen (NH_x-N) and oxidized-nitrogen (NO_x-N) of leachates collected from MYT Landfill (empty bar) and PPV Landfill (solid bar) from April 1994 to February 1995. Values shown are the means of 4 replicates. Vertical bars denote LSD by the Tukey's Honestly Significant Difference Test at $P = 0.05$.

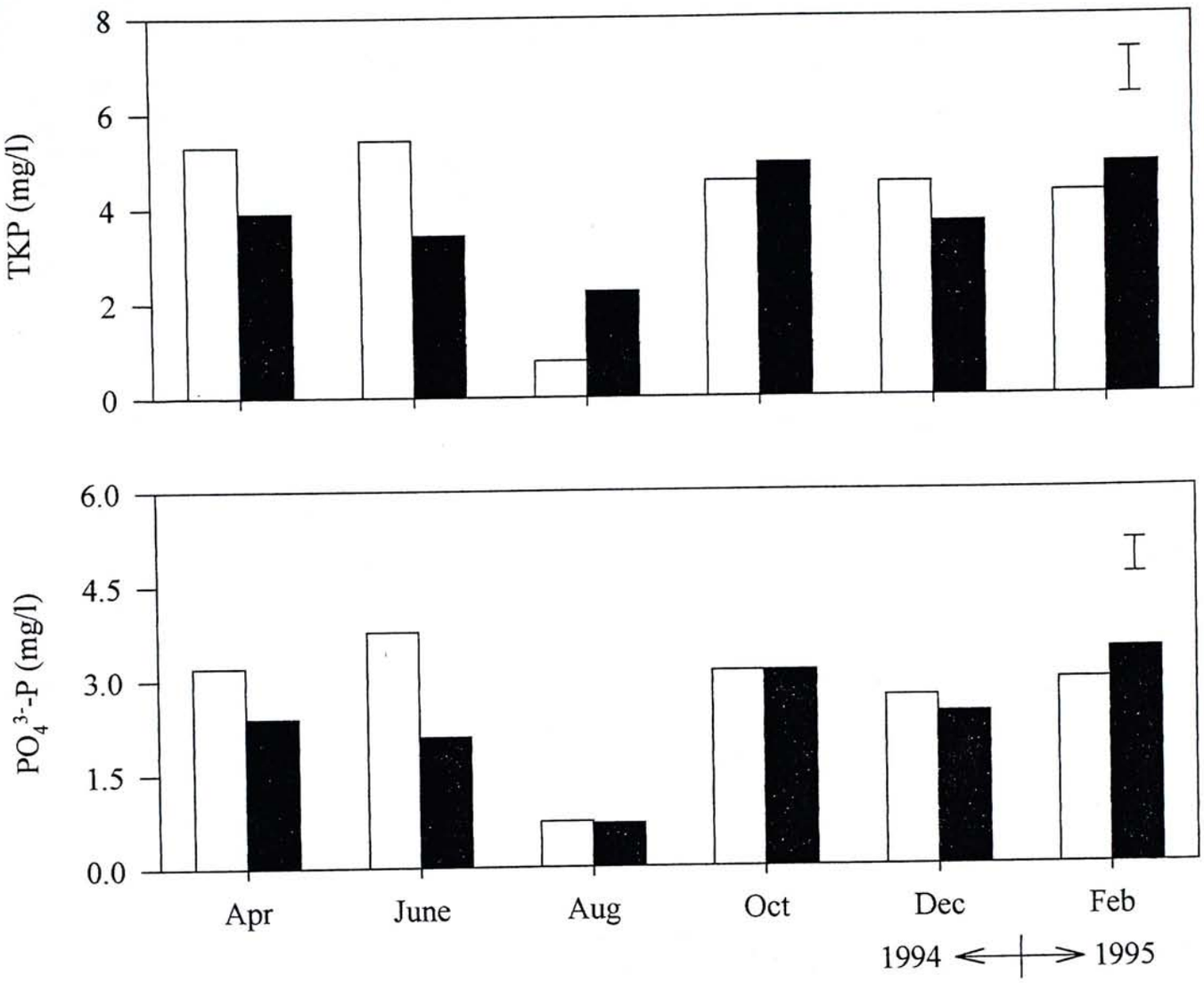


Fig. 2.5 Concentrations of total Kjeldahl phosphorus (TKP) and orthophosphate-phosphorus (PO_4^{3-}-P) of leachates collected from MYT Landfill (empty bar) and PPV Landfill (solid bar) from April 1994 to February 1995. Values shown are the means of 4 replicates. Vertical bars denote LSD by the Tukey's Honestly Significant Difference Test at $P = 0.05$.

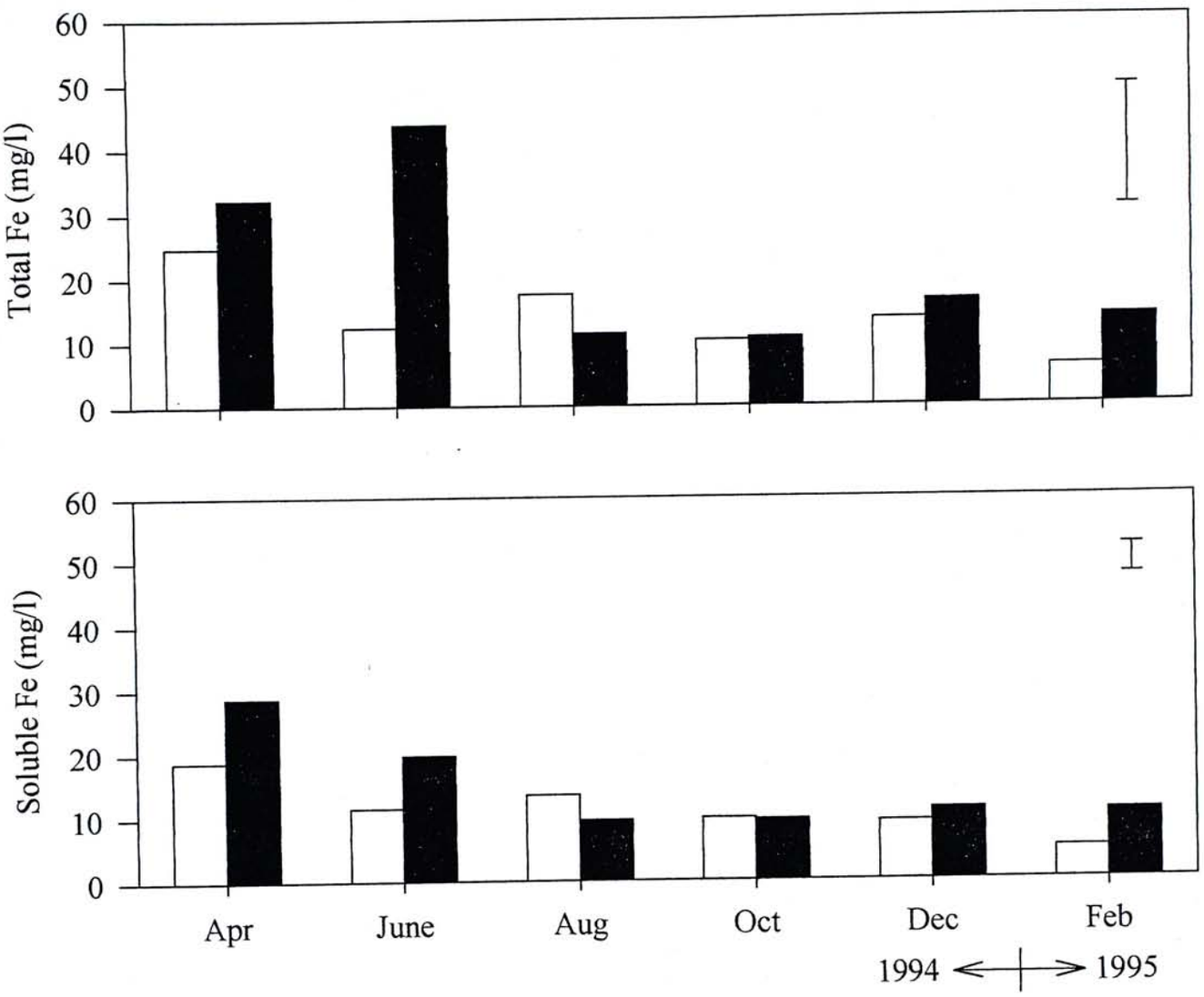


Fig. 2.6 Concentrations of total and soluble iron of leachates collected from MYT Landfill (empty bar) and PPV Landfill (solid bar) from April 1994 to February 1995. Values shown are the means of 4 replicates. Vertical bars denote LSD by the Tukey's Honestly Significant Difference Test at $P = 0.05$.

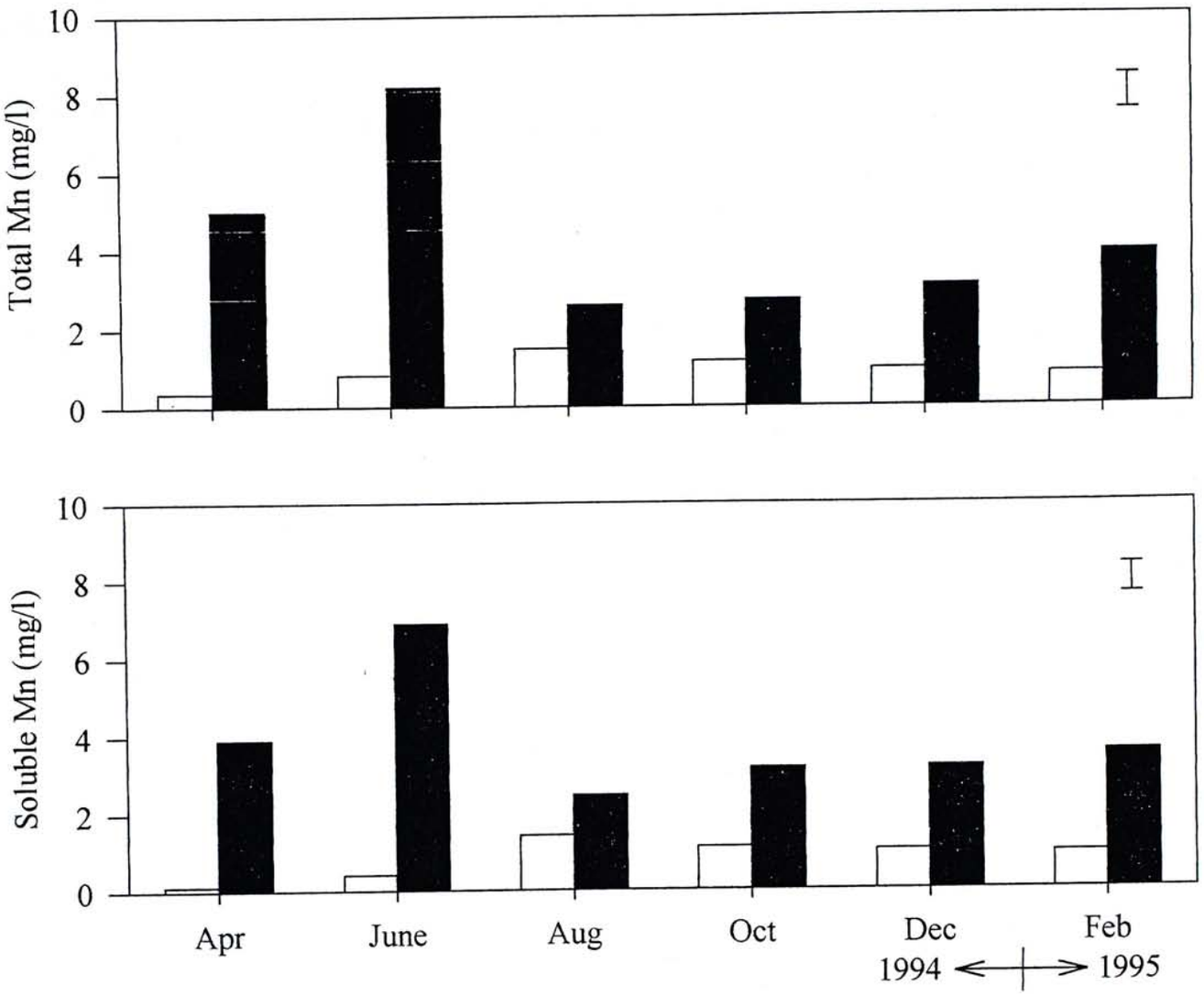


Fig. 2.7 Concentrations of total and soluble manganese of leachates collected from MYT Landfill (empty bar) and PPV Landfill (solid bar) from April 1994 to February 1995. Values shown are the means of 4 replicates. Vertical bars denote LSD by the Tukey's Honestly Significant Difference Test at $P = 0.05$.

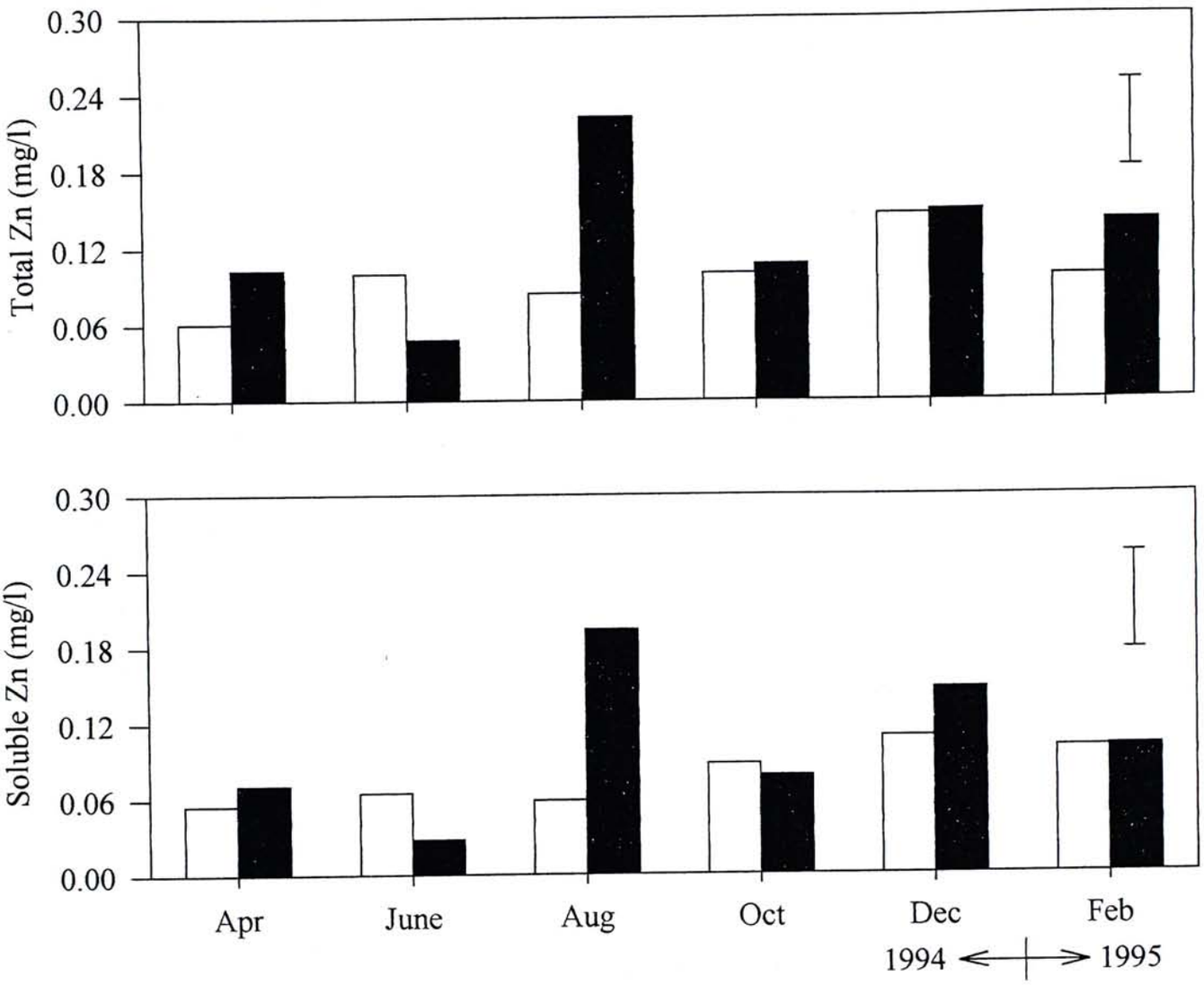


Fig. 2.8 Concentrations of total and soluble zinc of leachates collected from MYT Landfill (empty bar) and PPV Landfill (solid bar) from April 1994 to February 1995. Values shown are the means of 4 replicates. Vertical bars denote LSD by the Tukey's Honestly Significant Difference Test at $P = 0.05$.

Table 2.1 Chemical composition of leachates collected form the Ma Yau Tong Central (MYT) and Pillar Point Valley (PPV) Landfills from April 1994 to February 1995.

Parameters	MYT		PPV	
	Range[a]	Mean±SD[b]	Range[a]	Mean±SD[b]
pH	7.20-7.83	7.44±0.23	7.15-7.44	7.31±0.13
DO (mg/L)	1.95-4.37	3.49±0.95	0.88-3.71	1.94±0.96
EC (μS/cm)	5700-9860	8540±1470	8240-10800	9940±883
Salinity ($^{o}/_{oo}$)	5.00-8.00	6.23±1.06	7.00-8.75	7.71±0.72
TS (mg/L)	1710-3660	2350±652	2910-9440	4380±2320
COD (mg/L)	419-927	670±198	642-975	862±128
BOD (mg/L)	18.7-72.8	46.1±17.8	38.1-664	215±213
TKN (mg/L)	547-1270	917±263	684-971	770±106
NH_x-N (mg/L)	527-978	788±152	601-726	656±49
NO_x-N (mg/L)	0.11-0.76	0.49±0.26	0.04-0.70	0.23±0.02
TKP (mg/L)	0.77-5.45	4.12±1.72	2.23-4.93	3.84±1.01
PO_4^{3-}-P (mg/L)	0.73-5.30	2.75±1.05	0.69-3.44	2.36±0.92
Total metals (mg/L)				
Cd	ND	ND	ND	ND
Cr	ND	ND	ND	ND
Cu	ND	ND	ND	ND
Fe	6.02-24.7	14.2±6.6	10.6-43.8	21.3±15.7
Mn	0.37-1.48	0.94±0.41	2.61-5.03	4.24±2.07
Ni	ND	ND	ND	ND
Pb	ND	ND	ND	ND
Zn	0.06-0.15	0.10±0.04	0.05-0.22	0.14±0.06
Soluble metals (mg/L)				
Cd	ND	ND	ND	ND
Cr	ND	ND	ND	ND
Cu	ND	ND	ND	ND
Fe	4.95-18.8	11.3±4.7	9.55-28.7	14.9±7.5
Mn	0.13-1.42	0.84±0.45	2.47-6.91	3.86±1.61
Ni	ND	ND	ND	ND
Pb	ND	ND	ND	ND
Zn	0.06-0.11	0.08±0.03	0.03-0.19	0.11±0.06

[a] Range of means of six bimonthly samples

[b] Average value of six bimonthly samples

ND = not detectable

leachate.

Electrical conductivity (EC) is a numerical expression of the ability of an aqueous solution to carry an electric current. Solution of most inorganic acid, base and salt has high electrical conductivity. On the other hand, organic compounds which do not dissociate in aqueous solution has low conductivity. Total solids (TS) measure the amount of soluble and insoluble compounds in leachate. Leachates from both sites had very high value of EC and TS (Table 2.1). Leachate always has high value of conductivity, probably due to presence of high concentrations of calcium, magnesium, sodium and potassium, the salts of which dissolve easily in the water (Johansen and Carlson, 1976). On average, EC of the PPV leachate were 16.4% higher than those of the MYT leachate. The difference of TS between two leachates was even greater; TS of the PPV leachate was 24.1% to 157.7% higher than that of the MYT leachate (Fig. 2.2).

Leachate from the stabilized MYT Landfill had low BOD, which ranged from 18.7 - 72.8 mg/L (Table 2.1). COD was much higher (419 - 927 mg/L). More than 90% of the organic compounds in the leachate were non-biodegradable; BOD:COD ratio was as low as 0.02 to 0.11. BOD:COD is commonly used to predict the most suitable method for leachate treatment (Table 2.2). In terms of carbonaceous removal, leachate with a high BOD:COD ratio can be treated effectively by biological methods while physico-chemical methods are more suitable for leachate with low ratio (Chian and DeWalle, 1976). The low strength MYT leachate may not be amenable to conventional biological treatment. There is potential problem of low settleability of sludge due to a lack of heterotrophic bacteria (Environmental Protection Agency, 1993), which results in the production of poor quality effluent in conventional biological treatment.

Table 2.2 Proposed relationship between BOD:COD, COD and age of landfill to expected efficiency of organic removal from leachate (Chian and DeWalle, 1976).

BOD:COD ratio	> 0.5	0.1-0.5	< 0.1
COD (mg/L)	10 000	500-10 000	< 500
Age	young	medium	old
Biological treatment	good	fair	poor
Physico-chemical treatment			
precipitation	poor	fair	fair
oxidation	poor	fair	fair
ozonation	poor	fair	fair
reverse osmosis	poor	good	good
activated carbon	poor	fair	good
ion-exchange	poor	fair	fair

BOD of the PPV leachate decreased gradually from April 1994 to February 1995 (Fig. 2.3), regardless of the volume of rainfall. BOD dropped from 664 mg/L to 38.1 mg/L, a level similar to that of the MYT leachate. This might be due to the fact that the quantities of waste disposed of in the PPV Landfill was decreasing after the opening of the big strategic WENT Landfill. On the other hand, the COD of PPV leachate was relatively constant (624 - 975 mg/L) when compared with BOD (Fig. 2.3). A constant amount of non-biodegradable fraction was present in the leachate irrespective of the reduction in biodegradable fraction. BOD:COD ratio of the PPV leachate decreased from 0.65 to 0.04, a change in properties from a young leachate (BOD:COD > 0.5) to an old one (BOD:COD < 0.1) within a year. This suggests that organic composition of leachate can change in a very short period of time. Due to the possible dramatic change of leachate properties of an operating landfill, treatment plant must be flexible to allow adjustment and modification of treatment process.

MYT Landfill was older in age and more stabilized in terms of organic content. However, the leachate produced had higher concentrations of various forms of nitrogen (TKN, NH_x-N and NO_x-N) than the PPV leachate (Fig. 2.4). Ammonia was the major nitrogen compounds present in both leachates; ammonia represented 76.9 - 98.6% and 74.8 - 92.8% of the nitrogen in the MYT and PPV leachates respectively. Due to the anaerobic conditions prevail inside the landfill, very small amount of nitrogen exists as oxidized form; NO_x-N concentrations in the MYT and PPV leachates were never above 0.80 mg/L. The higher content of oxidized N in the MYT leachate may be attributed to the higher nitrogen concentration and DO of the leachate (Table 2.1).

Even the BOD was not excessive in the MYT leachate and PPV leachate, receiving water of the leachates would still suffer from problem of oxygen depletion

due to high ammonia concentration. Theoretically, nitrification of every gram of ammoniacal-N consumes 4.6 g oxygen (Eqs. 4.1 and 4.3). For MYT and PPV leachates which had average NH_x-N concentration of 788 and 659 mg/L, 3620 and 3200 mg/L oxygen will be consumed respectively for complete nitrification. Ammonia toxicity is another problem of concern. Acute toxicity effect for salmonid and non-salmonid fish species was reported for 0.1 to 10 mg/L unionized ammonia (Environmental Protection Agency, 1993). This is equivalent to 14.7 to 1470 mg/L NH_x-N at 25°C and pH 7. For receiving water with higher temperature and pH, concentration of unionized ammonia exists at higher concentration and toxic effect occurs at lower ammonia concentration. Ammoniacal-N concentrations of MYT and PPV leachates were within the toxic levels and the leachates were likely to be toxic to aquatic life in the receiving water.

Both leachates contained very low phosphorus concentration, mostly less than 5 mg/L of total phosphorus (Fig. 2.5). Sixty to seventy percent existed as orthophosphate, except for August which was 95% in the MYT leachate and 31% in the PPV leachate. The exceptionally high TS content of the PPV August sample may explain the higher insoluble fraction of phosphorus in the leachate. Low phosphorus level is found in acetogenic and methanogenic leachates (Ehrig, 1989). When biological methods are employed in treating leachate, phosphate is always added in order to maintain better growth of microorganisms.

Heavy metal concentrations were low. Cadmium, chromium, copper, nickel and lead concentrations were below detectable limits by flame atomic absorption spectrophotometry. Only iron, manganese and zinc were found at significant levels (Table 2.1). Iron was the most abundant metals in the leachates; 6.02 - 24.7 and 10.6-43.8 mg/L total iron were found in the MYT and PPV leachates respectively (Fig.

2.6). About 80% of iron were present in soluble forms. Manganese was the second most abundant metal; 0.37 - 1.48 and 2.61 - 5.03 mg/L total manganese were found in the MYT and PPV leachates respectively (Fig. 2.7). About 90% were in the soluble fraction. Similar concentration of zinc was found in the two leachates; 0.06 - 0.15 mg/L in the MYT leachate and 0.05 - 0.22 mg/L in the PPV leachate (Fig. 2.8). About 80% was soluble. On average, concentrations of iron, manganese and zinc in the PPV leachate were higher than those in the MYT leachate. As PPV is still an active site, leachate from newly deposited wastes attributed to the higher concentrations of heavy metals.

It seems to be a common phenomenon that iron, manganese and zinc are more abundant than other metals in leachate. The higher levels of iron and zinc were in of line with the leachate quality study of six Norway landfills and two USA landfills (Johansen and Carlson, 1976). Another study of two Hong Kong landfills (Gin Drinkers Bay and Tseung Kwan O) had similar results that concentrations of iron, manganese and zinc were higher than other metals (Chu *et al.*, 1994). Anaerobic condition in a landfill favors the formation of Fe^{2+} and Mn^{2+} which are more soluble than their oxidized form (Qasim and Chiang, 1994). Therefore, iron and manganese are more readily to dissolve in leachate under anaerobic condition. Concentration of metals in leachate is not only determined by the amount of metals in the wastes, but also by other processes such as precipitation, sorption, ion exchange and chelation. For instance, organic compounds which contain nitrogen, oxygen and sulfur can form soluble complex with metals and increase metal solubility (Andreottola and Cannas, 1992). On the other hand, other processes such as precipitation, adsorption and chelation reduce metal availability in leachate (Bagehi, 1987; Qasim and Chiang, 1994). In neutral pH condition that was found in the MYT and PPV leachates, metals

were more likely to form insoluble precipitates with sulfide, phosphate, carbonate, hydroxide and oxide (Qasim and Chiang, 1994). Humic acids and fulvic acids could also form strong complexing ligands to retain metals in landfill (Andreottola and Cannas, 1992).

Characterization of the organic constituents in leachate was done for samples collected in August 1994 and February 1995. Leachate contains a mixture of compounds which are the intermediate or end products of carbohydrates, lipids and proteins under anaerobic degradation (Fig. 2.9). Organic composition of leachate affects the performance of treatment process. For instance, lipids and oils enhance the growth of filamentous bacterium, *Microthrix parvicella*, which causes serious bulking problems and settling problem in the activated sludge process (Raunkijær *et al.*, 1994).

Lipids were not detected in both landfill leachates from the two samplings. Carbohydrates were present in higher concentration than proteins (Table 2.3). February samples had lower concentrations of carbohydrates and proteins than August samples. This agrees with the change of BOD levels of the leachates, which decreased for 59.6% for MYT and 80.7% for PPV during the same period. Proteins in wastes are the major source of ammonia of leachate. Nitrogen in proteins eventually reduces to ammonia under anaerobic condition of a landfill. Unlikely ammonia which is readily to dissolve in water, proteins will be retained in a landfill. Protein concentrations of MYT and PPV leachates were low even though ammonia concentrations were higher than 500 mg/L (Fig. 2.4). The small difference between TKN and NH_x-N concentrations (Table 2.1) reveals that only small amount of nitrogen existed as organic form in leachate.

Table 2.3 Concentrations of carbohydrates, proteins and lipids in the MYT and PPV Landfill leachates in August 1994 and February 1995. Values shown are the means and standard deviations of 4 replicates.

	Carbohydrates (mg/L)	Proteins (mg/L)	Lipids (mg/L)
MYT			
Aug 1994	14.3±1.3	0.54±0.03	ND
Feb 1995	5.25±0.32	0.30±0.01	ND
PPV			
Aug 1994	36.8±2.7	1.04±0.09	ND
Feb 1995	6.02±0.57	0.37±0.01	ND

ND = not detectable

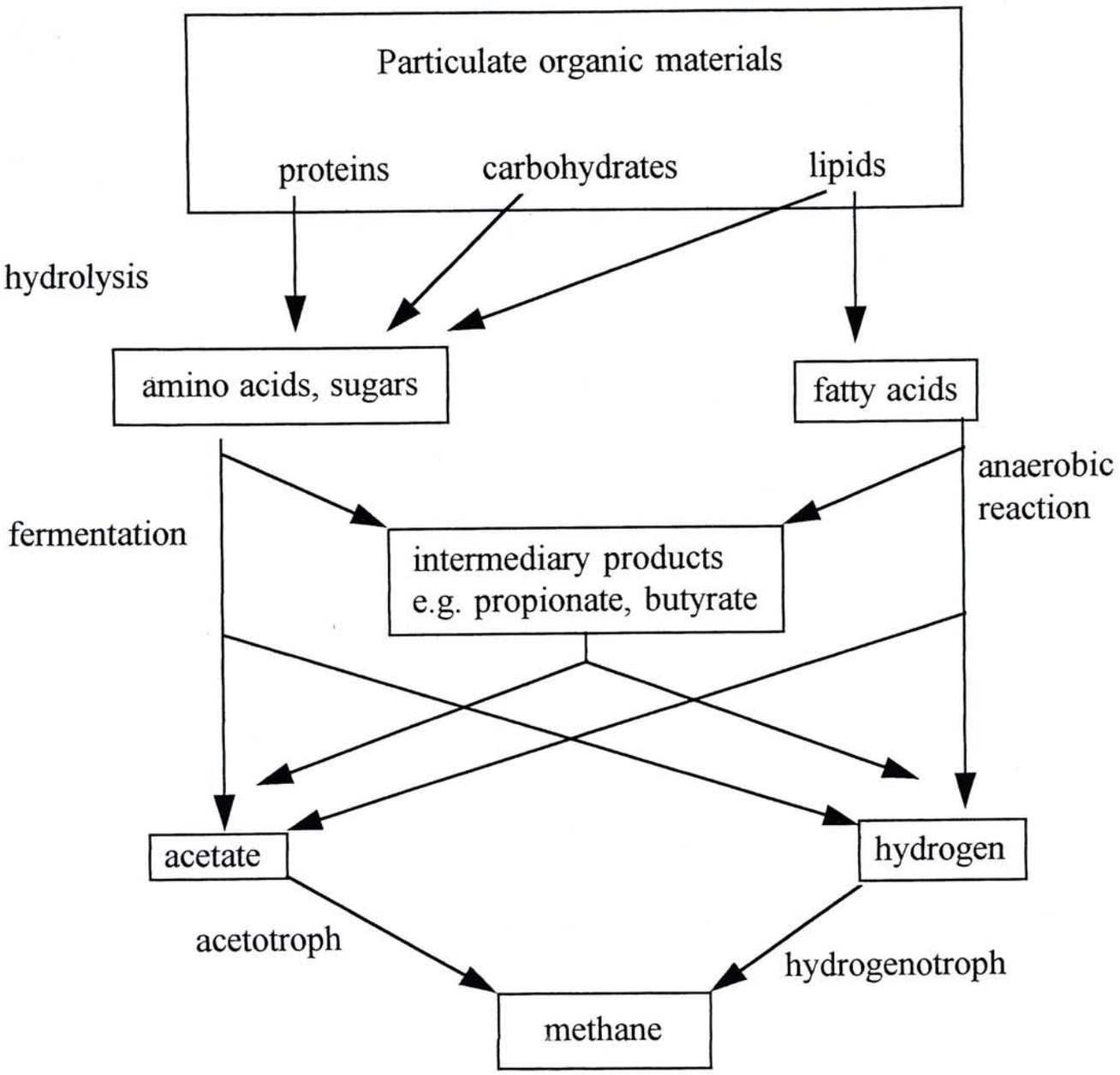


Fig. 2.9 Proposed degradation scheme for the organic fraction in a sanitary landfill (Lema *et al.*, 1988).

The concentrations of carbohydrates, proteins and lipids of the leachates are converted to COD unit using the following conversion terms based on assumed average composition: 1.13 mg COD /mg carbohydrates ($C_{10}H_{18}O_9$), 1.20 mg COD/mg proteins ($C_{14}H_{12}O_7N_2$) and 2.03 mg COD/mg lipid ($C_8H_6O_2$) (Raunkijær *et al*, 1994). Although there were differences in concentration, similar level of carbohydrates (as percentage of COD) were found in the August samples and were comparable to leachates from other landfills except Yuggesehi Landfill (Table 2.4). However, when degradability (as revealed by BOD:COD ratio) of MYT and PPV leachates further decreased to lower levels in February, carbohydrates only represented less than 1% of COD. Protein contents of MYT and PPV leachates only accounted for very small amount of COD (< 0.20%) for both the August and February samples. These were also much lower than leachates from other landfills (Table 2.4).

In domestic wastewater, carbohydrates, proteins and lipids represent higher percentage of organic compounds than those of landfill leachate (Table 2.4). Leachates of MYT and PPV had BOD:COD ratio of only 0.02 to 0.24. This is not unexpected as carbohydrates, proteins and lipids represented only small proportion of organic carbon in these two leachates. Even leachates with BOD:COD ratio higher than 0.5 as that of Brånådalen, Isi I, Grønmo, Taranrød and Yuggesehi Landfills (Table 2.4), lower proportion of COD was in the form of as carbohydrates when compared with domestic wastewater. Other organic compounds such as carboxylic acids, humic acids and fulvic acids attribute to most of COD in landfill leachate.

2.3.2 Temporal variation of leachate quality

Large temporal variation of leachate quality causes problems in treatment process. This kind of variation can be reflected by coefficients of variation of

Table 2.4 Organic contents of landfill leachate and domestic wastewater in terms of concentrations of carbohydrates, proteins and lipids and their concentrations expressed as percentage of COD. BOD, COD and BOD:COD ratio of the wastewater were also shown.

Wastewater	Carbohydrates		Proteins		Lipids		BOD	COD	BOD:
	conc. (mg/L)	% (as COD)	conc. (mg/L)	% (as COD)	conc. (mg/L)	% (as COD)	(mg/L)	(mg/L)	COD
Landfill leachate									
MYT (Aug)[a]	14.3	3.85	0.54	0.16	ND	-	46.3	419	0.11
MYT (Feb)[a]	5.25	0.70	0.30	0.04	ND	-	18.7	842	0.02
PPV (Aug)[a]	36.8	4.74	1.04	0.14	ND	-	198	877	0.24
PPV (Feb)[a]	2.70	0.37	0.37	0.05	ND	-	38.1	886	0.04
Brånådalen[b]	37.0	3.87	181	20.1	-	-	870	1,080	0.81
Isi I[b]	57.0	7.81	88.0	12.8	-	-	590	825	0.72
Grønmo[b]	24.0	5.77	-	-	-	-	320	470	0.68
Taranrød[b]	113	3.70	-	-	-	-	2,300	3,455	0.67
Yuggesehi[b]	54.0	0.65	144	1.83	-	-	5,250	9,425	0.56
Isi II[b]	6.00	6.16	-	-	-	-	50	110	0.45
Domestic wastewater									
Lyngby[c]	-	12.0	-	8.0	-	10.0	-	-	-
Tokyo[d]	1.00-18.0	6.0	19.0-42.0	12.0	42.0-54.0	19.0	-	242-270	-
Aalborg[e]	14.6-148	18.0	33.9-71.4	28.0	-	31.0	-	230-800	-

ND = not detectable
a Present study
b Johansen and Carlson, 1976
c Henze, 1992
d Tanaka *et al.*, 1991
e Raunkijær *et al.*, 1994

samplings of different months (Table 2.5). Variation of leachate quality was not site specific because some parameters showed higher variation in the MYT leachate and some in PPV leachate. Results of present study were compared with those of another study on two local landfills, Gin Drinkers Bay and Tseung Kwan O (Chu *et al.*, 1994). Since these two studies were carried out at different periods with different rainfall patterns, coefficients of variation of these two studies cannot be compared directly. However, in both studies, great variations were found in NO_x^--N, TKP, PO_4^{3-}-P, Mn and Zn concentrations when compared with other parameters (Table 2.5). Higher variation of COD was found in MYT, Gin Drinkers Bay and Tseung Kwan O leachates. For the present study, DO, TS, BOD and Fe concentration also exhibited high degree of variation ($\geq$ 30%) when compared with other parameters.

As BOD of leachate is affected by both biological processes and dilution effect of rainfall, its value also exhibited a larger variation than other parameters. For PPV leachate, the large variation was due to a gradual reduction of BOD during the sampling period (Fig. 2.3).

Oxidized nitrogen and phosphorus (TKP and PO_4^{3-}) had large temporal variation, despite their low concentrations in the leachates. Variation of their concentrations does not exhibit great influence on leachate treatment plant. The only possible problem is phosphorus deficiency when biological treatment is used for leachate treatment. Phosphorus must be added accordingly for optimal efficiency.

High coefficients of variation were found in DO and TS of the PPV leachate. When aerobic biological method is used for leachate treatment, aeration rate has to be adjusted accordingly to fulfill the requirement of oxygen. Leachate with high variation of TS concentration requires treatment flexible to handle episode of high solid content in the leachate. These control measures include the extension of

Table 2.5 Variability of chemical composition of leachates collected bimonthly from the Ma Yau Tong Central (MYT), Pillar Point Valley (PPV) Landfills from April 1994 to February 1995 and the Gin Drinkers Bay (GDB), Tseung Kwan O (TKO) Landfills from March 1990 to January 1991.

Parameters	Coefficient of variation (%)			
	MYT (Apr 94-Feb 95)[a]	PPV (Apr 94-Feb 95)[a]	GDB (Mar 90-Jan 91)[b]	TKO (Mar 90-Jan 91)[b]
pH	3.03	1.72	4.8	3.9
DO	27.1	49.7	-	-
EC	17.3	8.88	42	32
Salinity	15.9	9.35	28	29
TS	27.7	53.1	38	30
COD	30.0	14.9	64	54
BOD	38.5	99.0	-	-
TKN	22.9	13.7	59	40
NH_x-N	19.2	7.42	59	38
NO_x-N	52.3	101	69	66
TKP	41.4	26.0	48	80
PO_4^{3-}-P	38.2	39.1	48	87
Total metals				
Fe	46.3	73.4	44	48
Mn	43.7	48.1	71	95
Zn	35.4	39.6	36	91
soluble metals				
Fe	41.3	50.6	-	-
Mn	54.0	39.1	-	-
Zn	41.1	54.7	-	-

[a] from six bimonthly samples, (present study)

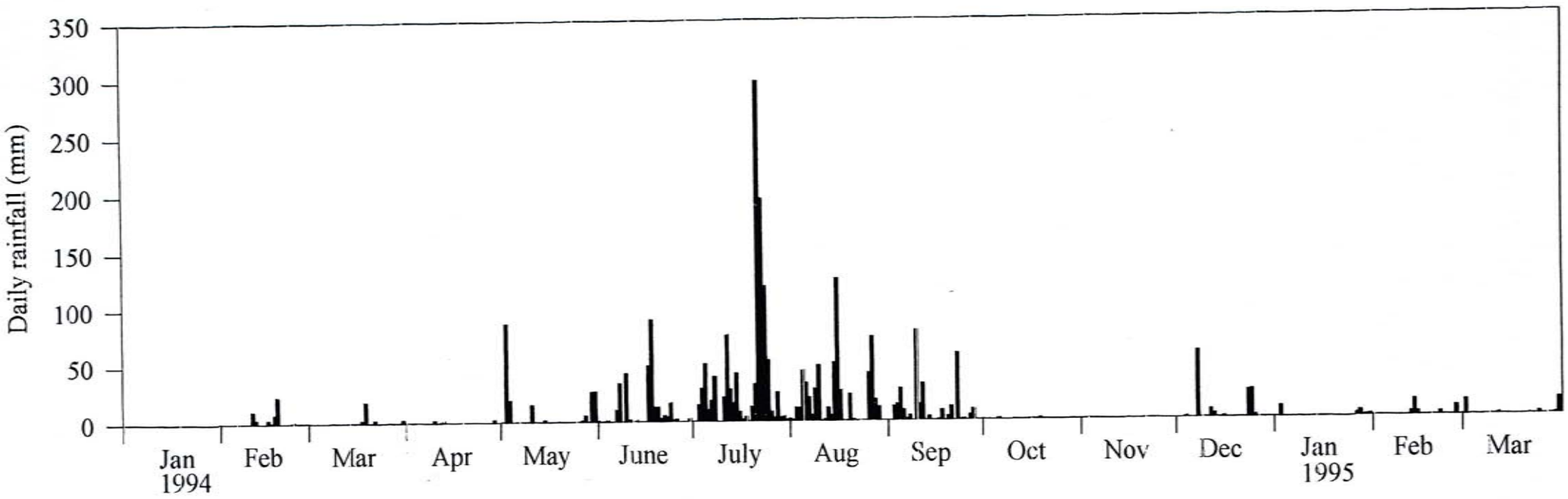
[b] from six bimonthly samples (Chu *et al.*, 1994)

retention time in sedimentation tank, occasional addition of chemicals to precipitate exceed solids and increase of capacity of handling settled solids.

2.3.3 Correlation of leachate quality and rainfall

Temporal variation of leachate quality depends on rainfall distribution throughout a year. Amount of water entering the site determines the volume of leachate produced which in turn affects leachate quality. The pollutant concentration depends on the degree of dilution and washing out due to increase of water flow. Higher rate of dilution decreases pollutant concentration in leachate while increase in washing out results in increase of concentration. Degree of correlation of leachate quality and rainfall is site specific and depends on site conditions such as decomposition, compaction of waste and flow of water inside the landfill. If the correlation of rainfall and pollutant concentration can be established, rainfall record can provide very useful information for predicting the leachate quality. Rainfall record can be obtained easily from local observatory or by installing rain gauges on a landfill.

The rainfall pattern of Hong Kong is predominantly seasonal and subtropical. According to the Hong Kong Royal Observatory, the average annual rainfall from 1966 - 1990 is 2210 mm. More than 70% of the rainfall are found in May to September when it is wet and hot. In 1994, the annual rainfall was 2730 mm, which was 23.5% wetter than usual. The temporal difference of rainfall was great (Fig. 2.10). July was the wettest month in the sampling period in which 1150 mm rainfall was recorded, which was equivalent to 42.1% of the 1994 annual rainfall. The total rainfall of July and August was equivalent to 63.9% of the annual rainfall, which was twice the normal figures for the two months. On the other hand, other months within



monthly total rainfall (mm)													
trace	50.5	26.5	6.0	183.7	290.2	1147.2	597.6	298.9	2.2	0.2	122.6	21.1	33.1	32.4
percentage of total annual rainfall													
-	1.85	0.97	0.22	6.74	10.65	42.1	21.9	10.0	0.07	0.01	4.50	-	-	-
normal rainfall (mm)													
23.4	48.0	66.9	161.5	316.7	376.0	323.5	391.4	299.7	144.8	35.1	27.3	23.4	48.0	66.9
percentage of total annual rainfall													
1.06	2.17	3.02	7.29	14.3	16.9	14.6	17.7	13.5	6.54	1.59	1.23	1.06	2.17	3.02

Fig. 2.10 Rainfall distribution of Hong Kong from January 1994 to March 1995. Table below the distribution chart shows the total monthly rainfall, percentage of total annual rainfall of each month, normal rainfall and percentage of total annual rainfall of each month (Royal Observatory, 1994a-l and 1995a-c).

the sampling period were drier than usual except December.

Due to the higher rainfall in July and August, the 1-day to 50-day cumulative rainfall before the August sampling was much higher than the other samplings (Fig. 2.11). 50-day cumulative rainfall was 1550 mm and this value was about 5 - 50 times as high as the other months. The lowest rainfall was recorded in April. The recorded rainfall was less than 10 mm from 1 to 37 days before sampling.

Correlation between leachate quality and cumulative rainfall was studied and correlation coefficients are shown in Tables 2.6-2.12.

No significant correlation was found between cumulative rainfall and pH, DO or salinity of leachate from both landfills (Table 2.6). Significant negative correlation of EC and cumulative rainfall was found only in the MYT leachate. Significant correlation ($P < 0.05$) was obtained for cumulative rainfall of 1 day and 18 days or more. Correlation was highly significant ($P < 0.01$) for 40- to 50-day cumulative rainfall (Fig. 2.12). This may be due to a time lapse between rainwater entering the site and leachate reaching the point of sampling. Salinity, like EC, indicates the amount of inorganic ions in leachate. However, no significant correlation was observed for the MYT leachate. The difference in scale may be responsible for the discrepancy between these two parameters. In the MYT leachate, the range of EC recorded was 5760 to 9860 μS/cm while salinity only exhibited 3‰ difference (Table 2.1). The fluctuation in inorganic ion concentration could not be expressed fully by the scale of salinity. Correlation between cumulative rainfall and EC or salinity of the PPV leachate was poor (Table 2.6).

Rainfall played a significant effect on the TS of leachates from both landfills; higher rainfall increased the TS of leachate (Figs. 2.13 and 2.14). In the MYT leachate, 4- to 26-day cumulative rainfall had a more determining effect on TS than

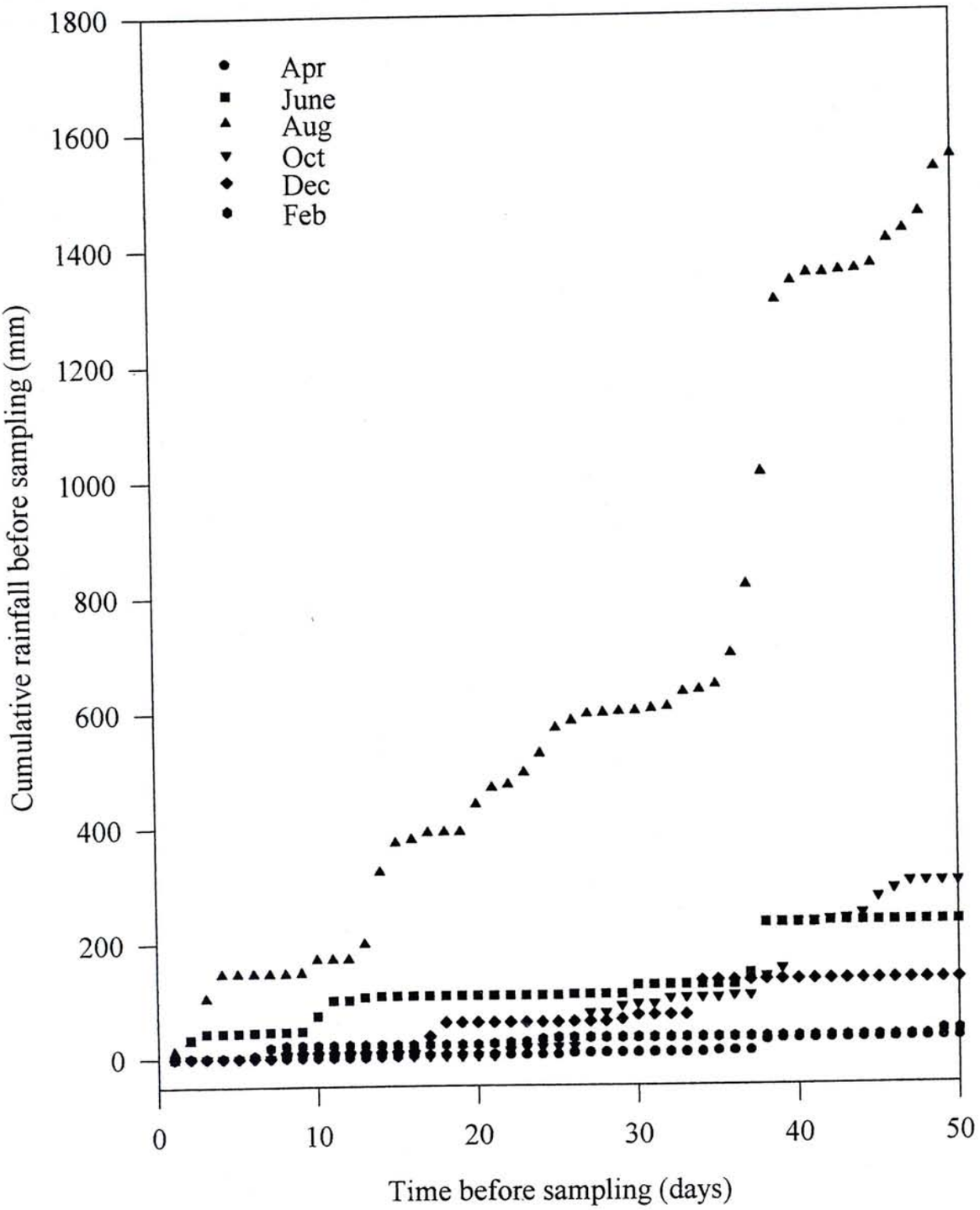


Fig. 2.11 The 1-day to 50-day cumulative rainfall before sampling.

Table 2.6 Correlation coefficient of 1-day to 50-day cumulative rainfall and pH, dissolved oxygen (DO), electrical conductivity (EC) or salinity of the MYT and PPV leachates.

Days before	pH		DO		EC		Salinity	
sampling	MYT	PPV	MYT	PPV	MYT	PPV	MYT	PPV
1	-0.512	0.540	0.446	-0.508	-0.927**	-0.062	-0.225	-0.359
2	-0.384	-0.116	-0.360	-0.600	-0.345	-0.803*	0.096	-0.695
3	-0.488	0.236	0.083	-0.628	-0.726	-0.476	-0.054	-0.559
4	-0.495	0.312	0.186	-0.610	-0.790	-0.378	-0.089	-0.505
5	-0.495	0.312	0.186	-0.610	-0.790	-0.378	-0.089	-0.505
6	-0.490	0.317	0.193	-0.595	-0.784	-0.376	-0.074	-0.494
7	-0.466	0.362	0.247	-0.520	-0.768	-0.344	-0.012	-0.429
8	-0.491	0.364	0.265	-0.527	-0.773	-0.335	-0.016	-0.405
9	-0.491	0.360	0.260	-0.529	-0.771	-0.340	0.015	-0.408
10	-0.485	0.275	0.145	-0.563	-0.712	-0.446	0.009	-0.478
11	-0.466	0.170	0.010	-0.578	-0.618	-0.563	0.052	-0.539
12	-0.467	0.171	0.010	-0.579	-0.618	-0.563	0.051	-0.539
13	-0.472	0.196	0.044	-0.585	-0.651	-0.533	0.030	-0.533
14	-0.491	0.316	0.198	-0.583	-0.772	-0.383	-0.050	-0.476
15	-0.493	0.342	0.232	-0.579	-0.795	-0.347	-0.067	-0.459
16	-0.492	0.341	0.236	-0.581	-0.797	-0.344	-0.069	-0.457
17	-0.521	0.335	0.244	-0.605	-0.809	-0.337	-0.092	-0.446
18	-0.548	0.324	0.247	-0.627	-0.814*	-0.334	-0.112	-0.436
19	-0.548	0.324	0.247	-0.627	-0.814*	-0.334	-0.112	-0.436
20	-0.542	0.344	0.269	-0.618	-0.827*	-0.310	-0.119	-0.426
21	-0.539	0.355	0.282	-0.610	-0.833*	-0.296	-0.120	-0.419
22	-0.547	0.369	0.286	-0.609	-0.842*	-0.285	-0.135	-0.426
23	-0.549	0.381	0.294	-0.605	-0.849*	-0.273	-0.144	-0.426
24	-0.543	0.393	0.031	-0.594	-0.853*	-0.259	-0.140	-0.414
25	-0.539	0.404	0.323	-0.586	-0.858*	-0.245	-0.141	-0.406
26	-0.535	0.403	0.323	-0.586	-0.858*	-0.244	-0.141	-0.406
27	-0.580	0.457	0.323	-0.588	-0.897*	-0.199	-0.224	-0.450
28	-0.580	0.457	0.323	-0.588	-0.897*	-0.199	-0.223	-0.450
29	-0.592	0.467	0.323	-0.590	-0.905*	-0.190	-0.242	-0.458
30	-0.602	0.452	0.300	-0.603	-0.898*	-0.212	-0.244	-0.475
31	-0.601	0.452	0.301	-0.603	-0.898*	-0.211	-0.244	-0.474
32	-0.607	0.459	0.300	-0.602	-0.903*	-0.204	-0.256	-0.480
33	-0.602	0.461	0.306	-0.600	-0.904*	-0.200	-0.254	-0.475
34	-0.645	0.444	0.310	-0.634	-0.911*	-0.194	-0.286	-0.458
35	-0.640	0.444	0.312	-0.633	-0.911*	-0.193	-0.284	-0.456
36	-0.630	0.450	0.322	-0.625	-0.914*	-0.184	-0.278	-0.449
37	-0.611	0.442	0.320	-0.620	-0.906*	-0.196	-0.256	-0.448
38	-0.593	0.408	0.268	-0.634	-0.886*	-0.250	-0.237	-0.493
39	-0.572	0.433	0.308	-0.611	-0.897*	-0.213	-0.230	-0.462
40	-0.595	0.465	0.309	-0.608	-0.916**	-0.183	-0.278	-0.485
41	-0.594	0.465	0.310	-0.607	-0.916**	-0.182	-0.277	-0.483
42	-0.595	0.465	0.308	-0.608	-0.916**	-0.183	-0.279	-0.486
43	-0.595	0.466	0.309	-0.607	-0.916**	-0.182	-0.279	-0.486
44	-0.598	0.469	0.308	-0.607	-0.918**	-0.179	-0.285	-0.488
45	-0.605	0.479	0.308	-0.606	-0.924**	-0.169	-0.301	-0.496
46	-0.605	0.485	0.311	-0.603	-0.926**	-0.162	-0.306	-0.495
47	-0.607	0.489	0.312	-0.601	-0.928**	-0.157	-0.311	-0.497
48	-0.605	0.489	0.315	-0.600	-0.928**	-0.156	-0.308	-0.494
49	-0.598	0.493	0.326	-0.592	-0.928**	-0.151	-0.298	-0.482
50	-0.596	0.493	0.327	-0.591	-0.927**	-0.151	-0.296	-0.480

* Correlation is significant at $P < 0.05$.

** Correlation is significant at $P < 0.01$.

Table 2.7 Correlation coefficient of 1-day to 50-day cumulative rainfall and total solids (TS), COD or BOD of the MYT and PPV leachates.

Days before sampling	TS		COD		BOD	
	MYT	PPV	MYT	PPV	MYT	PPV
1	0.889*	0.995**	-0.671	0.097	0.023	-0.070
2	0.686*	0.518	-0.377	-0.646	0.624	0.036
3	0.911*	0.872	-0.586	-0.295	0.338	-0.003
4	0.930**	0.924**	-0.614	-0.199	0.256	-0.013
5	0.930**	0.924**	-0.614	-0.199	0.256	-0.013
6	0.929**	0.922**	-0.607	-0.204	0.247	-0.021
7	0.923**	0.922**	-0.576	-0.203	0.187	-0.060
8	0.917**	0.924**	-0.600	-0.221	0.150	-0.095
9	0.917**	0.922**	-0.599	-0.225	0.154	-0.094
10	0.904*	0.873*	-0.575	-0.319	0.254	-0.069
11	0.866*	0.793	-0.529	-0.434	0.355	-0.052
12	0.865*	0.793	-0.529	-0.434	0.355	-0.052
13	0.883*	0.821*	-0.545	-0.395	0.337	-0.046
14	0.926**	0.916**	-0.603	-0.229	0.231	-0.042
15	0.930**	0.933**	-0.613	-0.190	0.205	-0.042
16	0.932**	0.935**	-0.614	-0.186	0.203	-0.039
17	0.926**	0.938**	-0.645	-0.197	0.177	0.063
18	0.916**	0.933**	-0.673	-0.213	0.154	-0.087
19	0.916**	0.933**	-0.673	-0.213	0.154	-0.087
20	0.920**	0.944**	-0.672	-0.182	0.141	-0.081
21	0.921**	0.949**	-0.671	-0.168	0.132	-0.080
22	0.917**	0.952**	-0.679	-0.156	0.130	-0.087
23	0.916**	0.956**	-0.682	-0.143	0.126	-0.088
24	0.917**	0.960**	-0.678	-0.130	0.113	-0.089
25	0.918**	0.965**	-0.676	-0.114	0.104	-0.087
26	0.920**	0.966**	-0.673	-0.110	0.106	-0.082
27	0.892*	0.970**	-0.715	-0.062	0.109	-0.108
28	0.892*	0.970**	-0.715	-0.061	0.109	-0.108
29	0.884*	0.969**	-0.725	-0.053	0.108	-0.116
30	0.882*	0.963**	-0.731	-0.077	0.125	-0.120
31	0.882*	0.963**	-0.730	-0.077	0.124	-0.120
32	0.877*	0.962**	-0.736	-0.069	0.125	-0.123
33	0.879*	0.964**	-0.733	-0.063	0.121	-0.120
34	0.861*	0.956**	-0.776	-0.087	0.084	-0.159
35	0.863*	0.958**	-0.773	-0.083	0.085	-0.154
36	0.868*	0.963**	-0.765	-0.070	0.081	-0.145
37	0.881*	0.966**	-0.747	-0.072	0.093	-0.127
38	0.893*	0.957**	-0.725	-0.102	0.158	-0.092
39	0.900*	0.970**	-0.712	-0.059	0.130	-0.080
40	0.879*	0.968**	-0.732	-0.028	0.130	-0.095
41	0.880*	0.969**	-0.732	-0.027	0.129	-0.094
42	0.879*	0.968**	-0.732	-0.027	0.131	-0.095
43	0.879*	0.968**	-0.733	-0.026	0.131	-0.095
44	0.876*	0.968**	-0.735	-0.022	0.131	-0.097
45	0.868*	0.966**	-0.741	-0.012	0.131	-0.102
46	0.866*	0.966**	-0.742	-0.005	0.128	-0.103
47	0.863*	0.966**	-0.743	0.000	0.128	-0.104
48	0.864*	0.967**	-0.742	0.001	0.126	-0.103
49	0.868*	0.970**	-0.736	0.005	0.117	-0.102
50	0.869*	0.971**	-0.735	0.005	0.115	-0.101

* Correlation is significant at $P < 0.05$.

** Correlation is significant at $P < 0.01$.

Table 2.8 Correlation coefficient of 1-day to 50-day cumulative rainfall and total Kjeldahl nitrogen (TKN), ammoniacal-N (NH_x-N) or oxidized nitrogen (NO_x-N) of the MYT and PPV leachates.

Days before sampling	TKN		NH_x-N		NO_x-N	
	MYT	PPV	MYT	PPV	MYT	PPV
1	-0.727	-0.174	-0.873*	-0.374	-0.736	-0.245
2	-0.201	-0.450	-0.484	-0.612	-0.178	-0.397
3	-0.524	-0.350	-0.761	-0.531	-0.529	-0.374
4	-0.582	-0.312	-0.798	-0.492	-0.593	-0.354
5	-0.582	-0.312	-0.798	-0.492	-0.593	-0.354
6	-0.580	-0.329	-0.797	-0.495	-0.584	-0.336
7	-0.580	-0.392	-0.794	-0.500	-0.556	-0.254
8	-0.604	-0.419	-0.804	-0.522	-0.529	-0.251
9	-0.602	-0.420	-0.803	-0.523	-0.528	-0.253
10	-0.541	-0.441	-0.765	-0.558	-0.475	-0.293
11	-0.456	-0.477	-0.703	-0.596	-0.387	-0.318
12	-0.457	-0.477	-0.703	-0.596	-0.387	-0.318
13	-0.480	-0.453	-0.723	-0.580	-0.425	-0.325
14	-0.579	-0.364	-0.794	-0.511	-0.556	-0.319
15	-0.598	-0.343	-0.805	-0.494	-0.582	-0.316
16	-0.599	-0.339	-0.805	-0.489	-0.586	-0.319
17	-0.624	-0.343	-0.815	-0.504	-0.571	-0.338
18	-0.643	-0.351	-0.821	-0.521	-0.549	-0.354
19	-0.643	-0.351	-0.821	-0.521	-0.549	-0.354
20	-0.651	-0.334	-0.826*	-0.504	-0.571	-0.347
21	-0.655	-0.329	-0.829*	-0.496	-0.580	-0.340
22	-0.665	-0.321	-0.838*	-0.499	-0.588	-0.335
23	-0.672	-0.314	-0.844*	-0.496	-0.597	-0.330
24	-0.674	-0.313	-0.845*	-0.488	-0.603	-0.319
25	-0.678	-0.306	-0.847*	-0.479	-0.613	-0.312
26	-0.676	-0.301	-0.845*	-0.474	-0.617	-0.315
27	-0.721	-0.256	-0.885*	-0.489	-0.650	-0.300
28	-0.721	-0.256	-0.885*	-0.489	-0.650	-0.300
29	-0.731	-0.247	-0.893*	-0.493	-0.654	-0.299
30	-0.728	-0.256	-0.893*	-0.510	-0.640	-0.309
31	-0.728	-0.256	-0.892*	-0.509	-0.641	-0.309
32	-0.774	-0.249	-0.897*	-0.510	-0.645	-0.306
33	-0.733	-0.246	-0.896*	-0.504	-0.650	-0.305
34	-0.763	-0.256	-0.903*	-0.529	-0.614	-0.331
35	-0.760	-0.253	-0.902*	-0.524	-0.618	-0.333
36	-0.758	-0.246	-0.900*	-0.512	-0.629	-0.328
37	-0.741	-0.249	-0.891*	-0.501	-0.635	-0.328
38	-0.705	-0.248	-0.876*	-0.505	-0.637	-0.350
39	-0.708	-0.231	-0.875*	-0.472	-0.663	-0.333
40	-0.732	-0.202	-0.894*	-0.477	-0.680	-0.322
41	-0.731	-0.202	-0.894*	-0.476	-0.681	-0.322
42	-0.732	-0.201	-0.894*	-0.477	-0.681	-0.322
43	-0.732	-0.201	-0.895*	-0.477	-0.682	-0.322
44	-0.735	-0.197	-0.897*	-0.477	-0.683	-0.320
45	-0.742	-0.187	-0.902*	-0.479	-0.688	-0.316
46	-0.744	-0.182	-0.904*	-0.476	-0.692	-0.313
47	-0.747	-0.178	-0.905*	-0.476	-0.695	-0.312
48	-0.746	-0.179	-0.905*	-0.474	-0.695	-0.311
49	-0.744	-0.184	-0.903*	-0.469	-0.696	-0.304
50	-0.744	-0.184	-0.902*	-0.467	-0.696	-0.304

* Correlation is significant at $P < 0.05$.

** Correlation is significant at $P < 0.01$.

Table 2.9 Correlation coefficient of 1-day to 50-day cumulative rainfall and total Kjeldahl phosphorus (TKP) or orthophosphate-P (PO_4^{3-}-P) of the MYT and PPV leachates.

Days before sampling	TKP		PO_4^{3-}-P	
	MYT	PPV	MYT	PPV
1	-0.970**	-0.738	-0.942**	-0.819*
2	-0.427	-0.746	-0.322	-0.751
3	-0.806	-0.859*	-0.741	-0.903*
4	-0.866*	-0.853*	-0.813*	-0.905*
5	-0.866*	-0.853*	-0.813*	-0.905*
6	-0.867*	-0.847*	-0.811*	-0.898*
7	-0.878*	-0.814*	-0.815*	-0.861*
8	-0.889*	-0.825*	-0.832*	-0.864*
9	-0.887*	-0.826*	-0.829*	-0.865*
10	-0.826*	-0.842*	-0.757	-0.874*
11	-0.734	-0.837*	-0.652	-0.859*
12	-0.734	-0.837*	-0.652	-0.859*
13	-0.762	-0.845*	-0.685	-0.871*
14	-0.866*	-0.848*	-0.809*	-0.892*
15	-0.885*	-0.844*	-0.833*	-0.892*
16	-0.887**	-0.846*	-0.836*	-0.894*
17	-0.896*	-0.863*	-0.854*	-0.905*
18	-0.898*	-0.877*	-0.864*	-0.911*
19	-0.898*	-0.877*	-0.864*	-0.911*
20	-0.909*	-0.869*	-0.876*	-0.907*
21	-0.915*	-0.864*	-0.882*	-0.904*
22	-0.919**	-0.857*	-0.885*	-0.900*
23	-0.924**	-0.851*	-0.889*	-0.897*
24	-0.929**	-0.844*	-0.895*	-0.891*
25	-0.934**	-0.838*	-0.901*	-0.887*
26	-0.934**	-0.839*	-0.901*	-0.889*
27	-0.943**	-0.800	-0.903*	-0.866*
28	-0.943**	-0.800	-0.903*	-0.866*
29	-0.944**	-0.792	-0.903*	-0.861*
30	-0.936**	-0.800	-0.894*	-0.867*
31	-0.937**	-0.800	-0.895*	-0.867*
32	-0.937**	-0.793	-0.894*	-0.862*
33	-0.939**	-0.793	-0.897*	-0.862*
34	-0.942**	-0.813*	-0.912*	-0.870*
35	-0.942**	-0.813*	-0.913*	-0.871*
36	-0.946**	-0.810	-0.917**	-0.870*
37	-0.944**	-0.816*	-0.913*	-0.875*
38	-0.923**	-0.827*	-0.885*	-0.889*
39	-0.937**	-0.815*	-0.902*	-0.881*
40	-0.938**	-0.787	-0.899*	-0.863*
41	-0.938**	-0.787	-0.900*	-0.863*
42	-0.938**	-0.786	-0.899*	-0.862*
43	-0.938**	-0.786	-0.899*	-0.862*
44	-0.937**	-0.783	-0.898*	-0.860*
45	-0.937**	-0.772	-0.896*	-0.852*
46	-0.938**	-0.768	-0.897*	-0.849*
47	-0.937**	-0.763	-0.897*	-0.846*
48	-0.938**	-0.764	-0.898*	-0.847*
49	-0.942**	-0.764	-0.902*	-0.846*
50	-0.943**	-0.765	-0.903*	-0.846*

* Correlation is significant at $P < 0.05$.

** Correlation is significant at $P < 0.01$.

Table 2.10 Correlation coefficient of 1-day to 50-day cumulative rainfall and total or soluble iron of the MYT and PPV leachates.

Days before sampling	Total Fe		Soluble Fe	
	MTY	PPV	MTY	PPV
1	0.220	-0.411	0.208	-0.381
2	0.070	0.381	0.180	-0.017
3	0.194	-0.028	0.236	-0.213
4	0.216	-0.126	0.240	-0.253
5	0.216	-0.126	0.240	-0.253
6	0.203	-0.127	0.227	-0.258
7	0.145	-0.153	0.165	-0.288
8	0.131	-0.175	0.137	-0.318
9	0.131	-0.170	0.138	-0.315
10	0.126	-0.064	0.153	-0.264
11	0.100	0.063	0.149	-0.206
12	0.100	0.063	0.149	-0.207
13	0.121	0.029	0.165	-0.215
14	0.181	-0.124	0.200	-0.269
15	0.193	-0.158	0.207	-0.280
16	0.198	-0.161	0.211	-0.279
17	0.201	-0.181	0.201	-0.300
18	0.201	-0.196	0.189	-0.319
19	0.201	-0.196	0.189	-0.319
20	0.208	-0.216	0.195	-0.322
21	0.209	-0.227	0.195	-0.325
22	0.203	-0.239	0.192	-0.336
23	0.203	-0.250	0.192	-0.341
24	0.201	-0.261	0.188	-0.344
25	0.203	-0.272	0.189	-0.347
26	0.208	-0.271	0.195	-0.342
27	0.183	-0.320	0.183	-0.386
28	0.183	-0.320	0.183	-0.386
29	0.177	-0.331	0.179	-0.397
30	0.172	-0.315	0.176	-0.395
31	0.173	-0.315	0.177	-0.395
32	0.169	-0.322	0.175	-0.401
33	0.172	-0.325	0.177	-0.399
34	0.171	-0.349	0.158	-0.429
35	0.176	-0.348	0.162	-0.425
36	0.182	-0.352	0.168	-0.420
37	0.191	-0.335	0.180	-0.401
38	0.203	-0.277	0.208	-0.362
39	0.216	-0.303	0.215	-0.361
40	0.200	-0.332	0.207	-0.385
41	0.201	-0.333	0.208	-0.384
42	0.200	-0.333	0.207	-0.385
43	0.200	-0.333	0.207	-0.385
44	0.198	-0.336	0.206	-0.388
45	0.192	-0.346	0.203	-0.396
46	0.191	-0.351	0.203	-0.398
47	0.190	-0.355	0.202	-0.401
48	0.191	-0.356	0.203	-0.400
49	0.191	-0.359	0.201	-0.399
50	0.192	-0.359	0.201	-0.398

* Correlation is significant at $P < 0.05$.

** Correlation is significant at $P < 0.01$.

Table 2.11 Correlation coefficient of 1-day to 50-day cumulative rainfall and total or soluble manganese of the MYT and PPV leachates.

Days before sampling	Total Mn		Soluble Mn	
	MTY	PPV	MTY	PPV
1	0.755	-0.424	0.637	-0.465
2	0.429	-0.448	0.108	0.451
3	0.656	-0.007	0.420	-0.037
4	0.686	-0.117	0.480	-0.153
5	0.686	-0.117	0.480	-0.153
6	0.685	-0.112	0.481	-0.150
7	0.685	-0.118	0.500	-0.158
8	0.698	-0.141	0.527	-0.178
9	0.697	-0.136	0.524	-0.173
10	0.666	-0.023	0.457	-0.054
11	0.614	0.117	0.372	0.094
12	0.615	0.117	0.372	0.094
13	0.629	0.076	0.393	0.050
14	0.685	-0.103	0.486	-0.140
15	0.693	-0.143	0.505	-0.182
16	0.692	-0.148	0.505	-0.187
17	0.705	-0.175	0.528	-0.213
18	0.714	-0.195	0.545	-0.230
19	0.714	-0.195	0.545	-0.230
20	0.717	-0.218	0.553	-0.254
21	0.719	-0.229	0.558	-0.267
22	0.729	-0.241	0.569	-0.275
23	0.735	-0.252	0.575	-0.286
24	0.735	-0.262	0.580	-0.298
25	0.735	-0.273	0.584	-0.311
26	0.733	-0.274	0.581	-0.312
27	0.780	-0.320	0.623	-0.346
28	0.780	-0.320	0.623	-0.346
29	0.780	-0.331	0.633	-0.354
30	0.792	-0.313	0.629	-0.333
31	0.791	-0.314	0.628	-0.334
32	0.797	-0.320	0.634	-0.338
33	0.795	-0.324	0.633	-0.343
34	0.809	-0.357	0.631	-0.371
35	0.806	-0.357	0.659	-0.372
36	0.802	-0.362	0.657	-0.380
37	0.789	-0.344	0.640	-0.366
38	0.772	-0.283	0.599	-0.306
39	0.766	-0.313	0.604	-0.342
40	0.790	-0.340	0.627	-0.362
41	0.789	-0.341	0.627	-0.362
42	0.790	-0.341	0.627	-0.362
43	0.790	-0.341	0.627	-0.362
44	0.793	-0.344	0.630	-0.364
45	0.800	-0.353	0.637	-0.370
46	0.802	-0.358	0.639	-0.375
47	0.804	-0.362	0.642	-0.379
48	0.803	-0.363	0.641	-0.380
49	0.800	-0.365	0.641	-0.385
50	0.799	-0.366	0.641	-0.385

* Correlation is significant at $P < 0.05$.

** Correlation is significant at $P < 0.01$.

Table 2.12 Correlation coefficient of 1-day to 50-day cumulative rainfall and total or soluble zinc of the MYT and PPV leachates.

Days before sampling	Total Zn		Soluble Zn	
	MTY	PPV	MTY	PPV
1	-0.248	0.795	-0.417	0.747
2	-0.160	0.047	-0.577	0.055
3	-0.232	0.511	-0.559	0.490
4	-0.240	0.603	-0.533	0.575
5	-0.240	0.603	-0.533	0.578
6	-0.241	0.603	-0.528	0.573
7	-0.246	0.621	-0.194	0.580
8	-0.194	0.649	-0.449	0.617
9	-0.195	0.645	-0.452	0.613
10	-0.194	0.549	-0.493	0.624
11	-0.180	0.423	-0.519	0.407
12	-0.180	0.423	-0.519	0.407
13	-0.192	0.459	-0.524	0.440
14	-0.222	0.605	-0.508	0.577
15	-0.227	0.636	-0.500	0.605
16	-0.226	0.640	-0.500	0.610
17	-0.175	0.666	-0.464	0.648
18	-0.122	0.685	-0.426	0.679
19	-0.122	0.685	-0.426	0.679
20	-0.137	0.700	-0.429	0.690
21	-0.144	0.708	-0.429	0.696
22	-0.146	0.711	-0.427	0.697
23	-0.150	0.716	-0.426	0.700
24	-0.157	0.724	-0.424	0.706
25	-0.165	0.732	-0.424	0.711
26	-0.169	0.732	-0.428	0.711
27	-0.171	0.731	-0.419	0.706
28	-0.171	0.731	-0.419	0.706
29	-0.166	0.732	-0.413	0.707
30	-0.154	0.718	-0.415	0.696
31	-0.155	0.718	-0.415	0.697
32	-0.155	0.717	-0.413	0.695
33	-0.159	0.721	-0.414	0.698
34	-0.075	0.750	-0.353	0.746
35	-0.080	0.751	-0.357	0.746
36	-0.094	0.756	-0.364	0.748
37	-0.118	0.748	-0.385	0.736
38	-0.156	0.701	-0.438	0.685
39	-0.180	0.725	-0.438	0.703
40	-0.181	0.722	-0.431	0.697
41	-0.181	0.723	-0.431	0.698
42	-0.181	0.721	-0.432	0.696
43	-0.182	0.722	-0.432	0.696
44	-0.182	0.721	-0.431	0.695
45	-0.182	0.719	-0.428	0.692
46	-0.184	0.720	-0.427	0.693
47	-0.184	0.720	-0.426	0.692
48	-0.186	0.722	-0.426	0.694
49	-0.190	0.728	-0.425	0.699
50	-0.190	0.730	-0.425	0.700

* Correlation is significant at $P < 0.05$.

** Correlation is significant at $P < 0.01$.

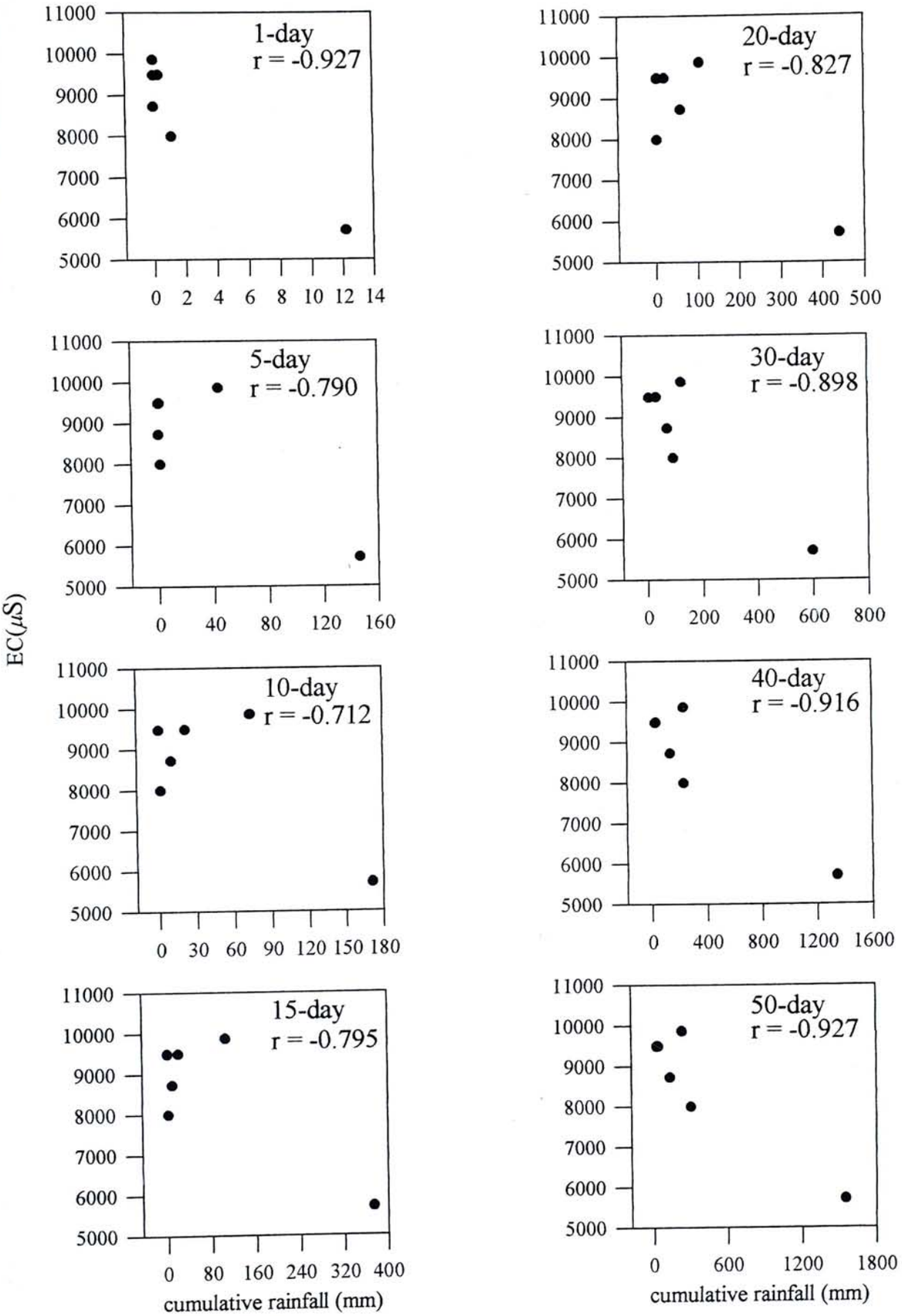


Fig. 2.12 The correlation of conductivity (EC) of Ma Yau Tong leachate to 1-day, 5-day, 10-day, 15-day, 20-day, 30-day, 40-day and 50-day cumulative rainfall.

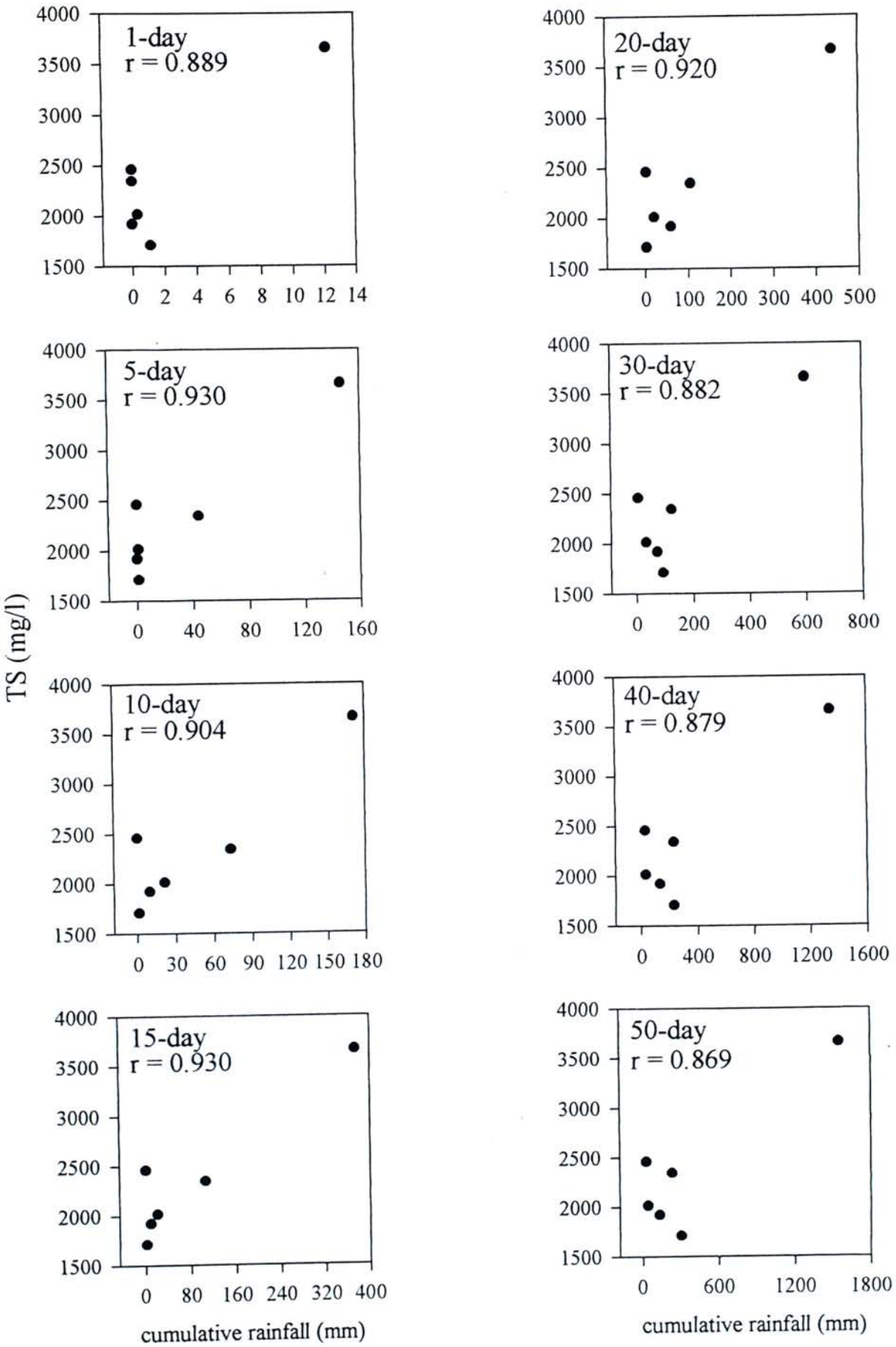


Fig. 2.13 The correlation of total solids (TS) of Ma Yau Tong leachate to 1-day, 5-day, 10-day, 15-day, 20-day, 30-day, 40-day and 50-day cumulative rainfall.

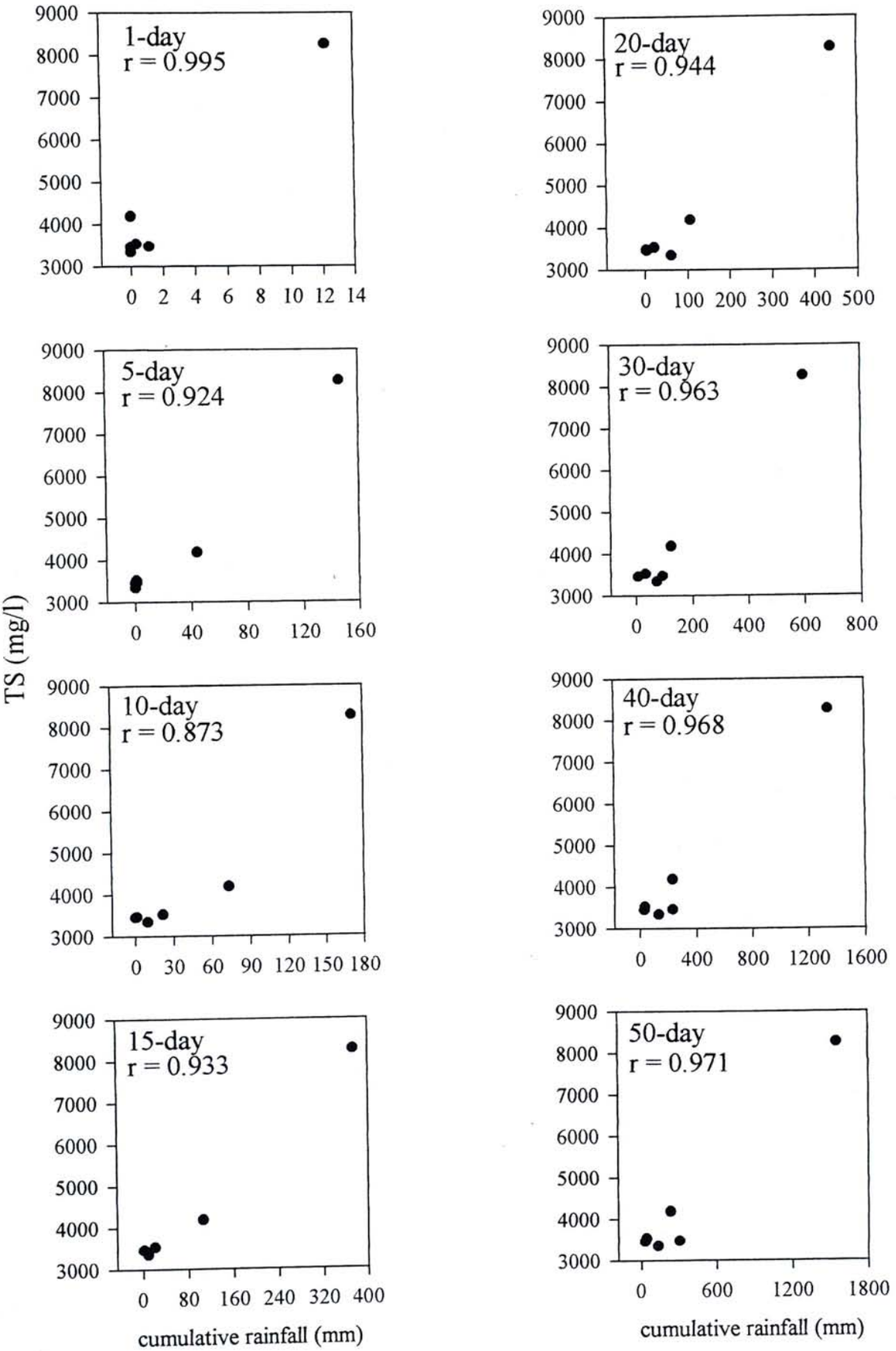


Fig. 2.14 The correlation of total solids (TS) of Pillar Point Valley leachate to 1-day, 5-day, 10-day, 15-day, 20-day, 30-day, 40-day and 50-day cumulative rainfall.

other duration (Table 2.7). In the PPV leachate, TS concentration showed significant correlation ($P < 0.01$) with 1- to 50-day cumulative rainfall. Increase in flow rate increase the chance of physical washout of the particulate matter in the younger landfill. During high rainfall period, leachate may contain very high solid concentration that the designed treatment operation cannot handle. The design of treatment plant should consider whether occasional high solid concentration in effluent is acceptable or temporarily additional step (e.g. chemical precipitation) is employed to remove excessive solids during high rainfall period. If the latter is chosen, rainfall record is a good indicator to predict when the additional treatment step is required.

No significant correlation was found between cumulative rainfall and COD or BOD in both sites (Table 2.7). For ammoniacal-N concentration in leachates, significant correlation was only obtained between MYT leachate and cumulative rainfall of 16 days or more (Table 2.8 and Fig. 2.15). Rainfall had little effect on the concentrations of TKN and NO_x-N. It seems that the levels of organic carbon and nitrogen were affected by other factors such as degradation of the fills instead of water flow alone. Parameters, such as TS, that are solely affected by infiltration, would show a stronger correlation with rainfall. Although no significant correlation could be found for COD, TKN and oxidized-N, negative correlation coefficients (Tables 2.7 and 2.8) indicate that they tended to be diluted by the increasing flow of water.

Strong correlation was found between phosphorus (TKP and orthophosphate) and cumulative rainfall (Figs. 2.16-19). Significant correlation ($P < 0.05$) was found almost every day from 1 to 50 days before sampling. For the MYT leachate, TKP showed a stronger correlation ($P < 0.01$) with 22-day to 50-day cumulative rainfall

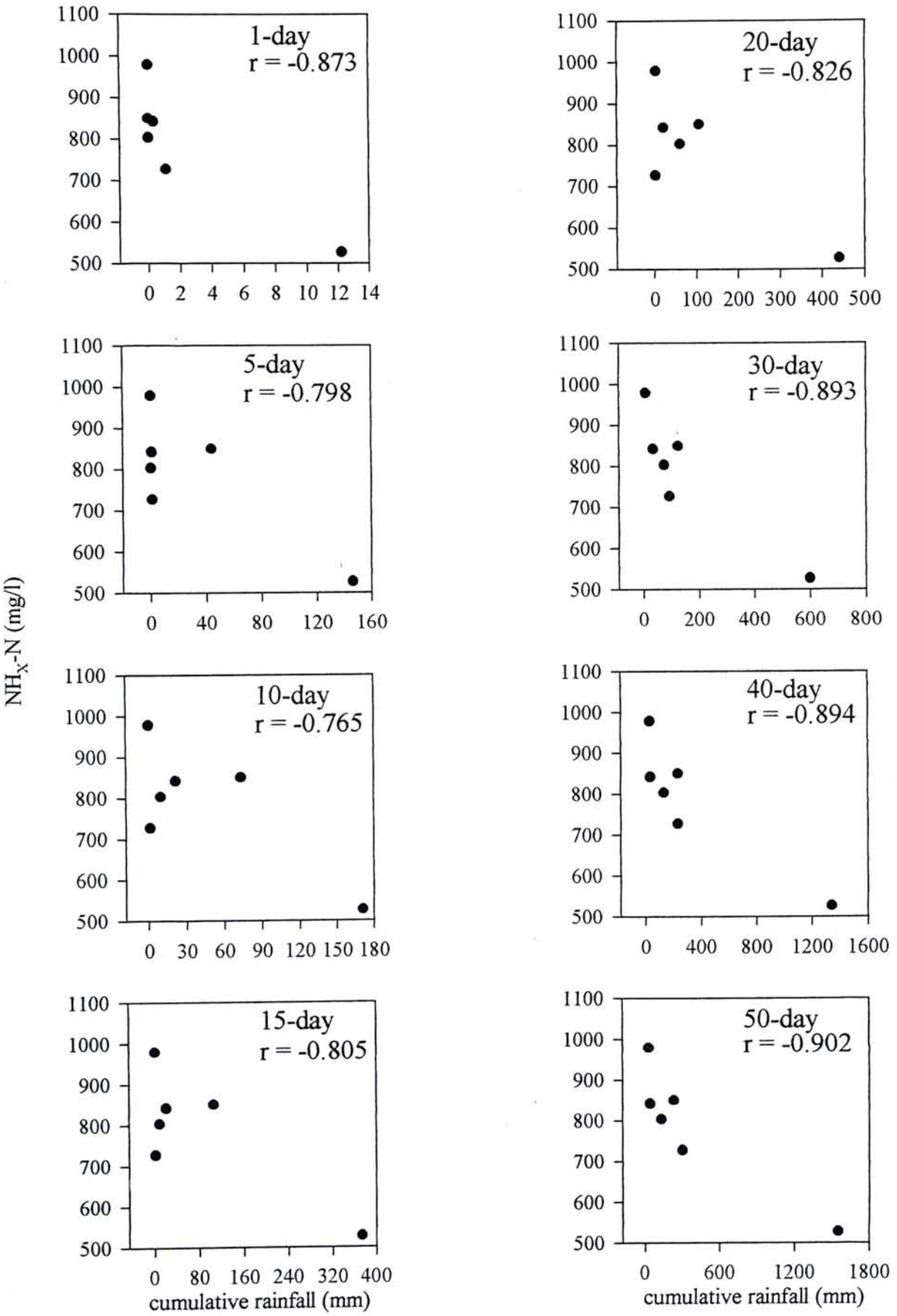


Fig. 2.15 The correlation of ammoniacal-N (NH_x-N) of the Ma Yau Tong Central leachate to 1-day, 5-day, 10-day, 15-day, 20-day, 30-day, 40-day and 50-day cumulative rainfall.

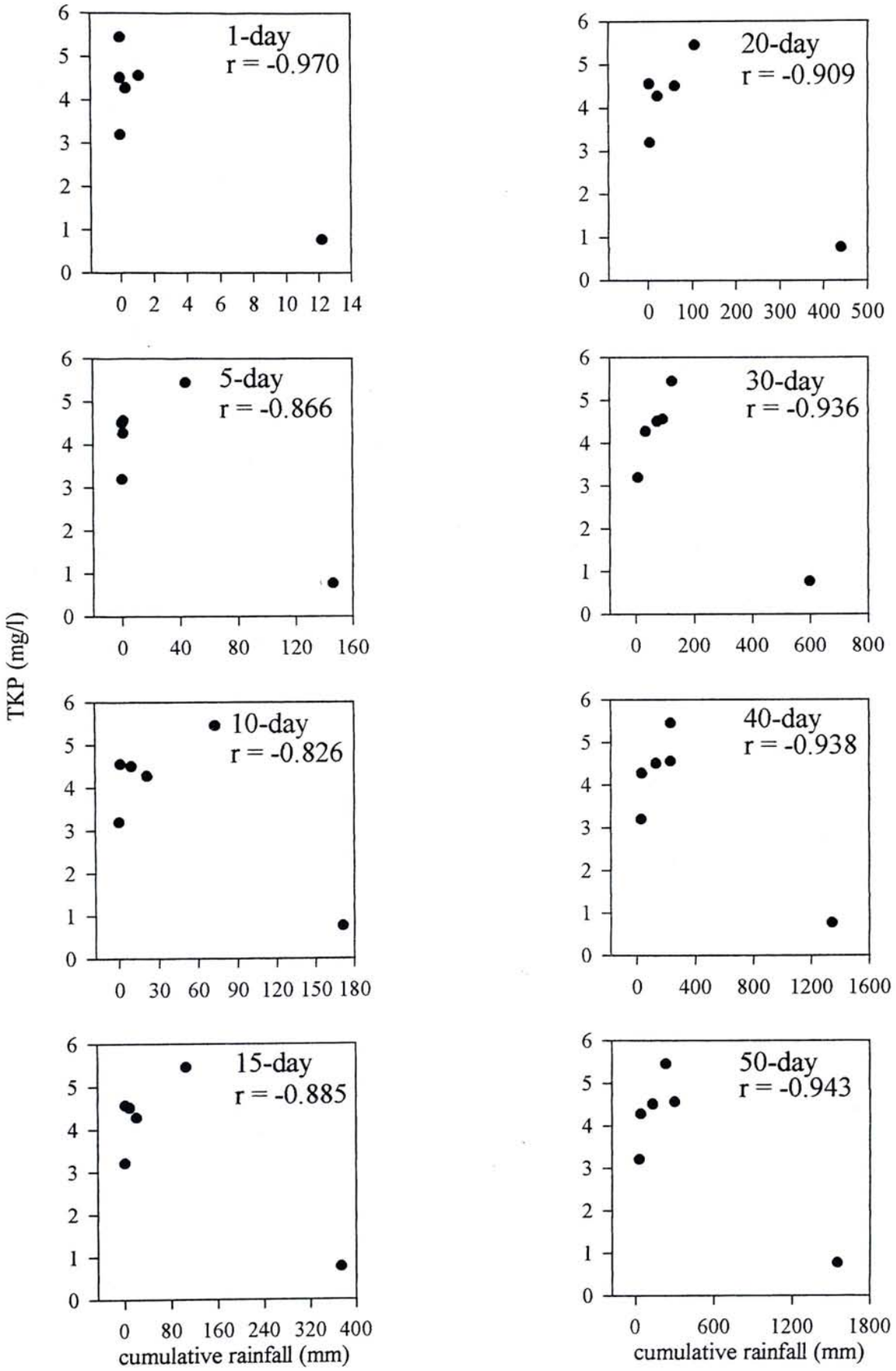


Fig. 2.16 The correlation of total Kjeldahl phosphorus (TKP) of the Ma Yau Tong Central leachate to 1-day, 5-day, 10-day, 15-day, 20-day, 30-day, 40-day and 50-day cumulative rainfall.

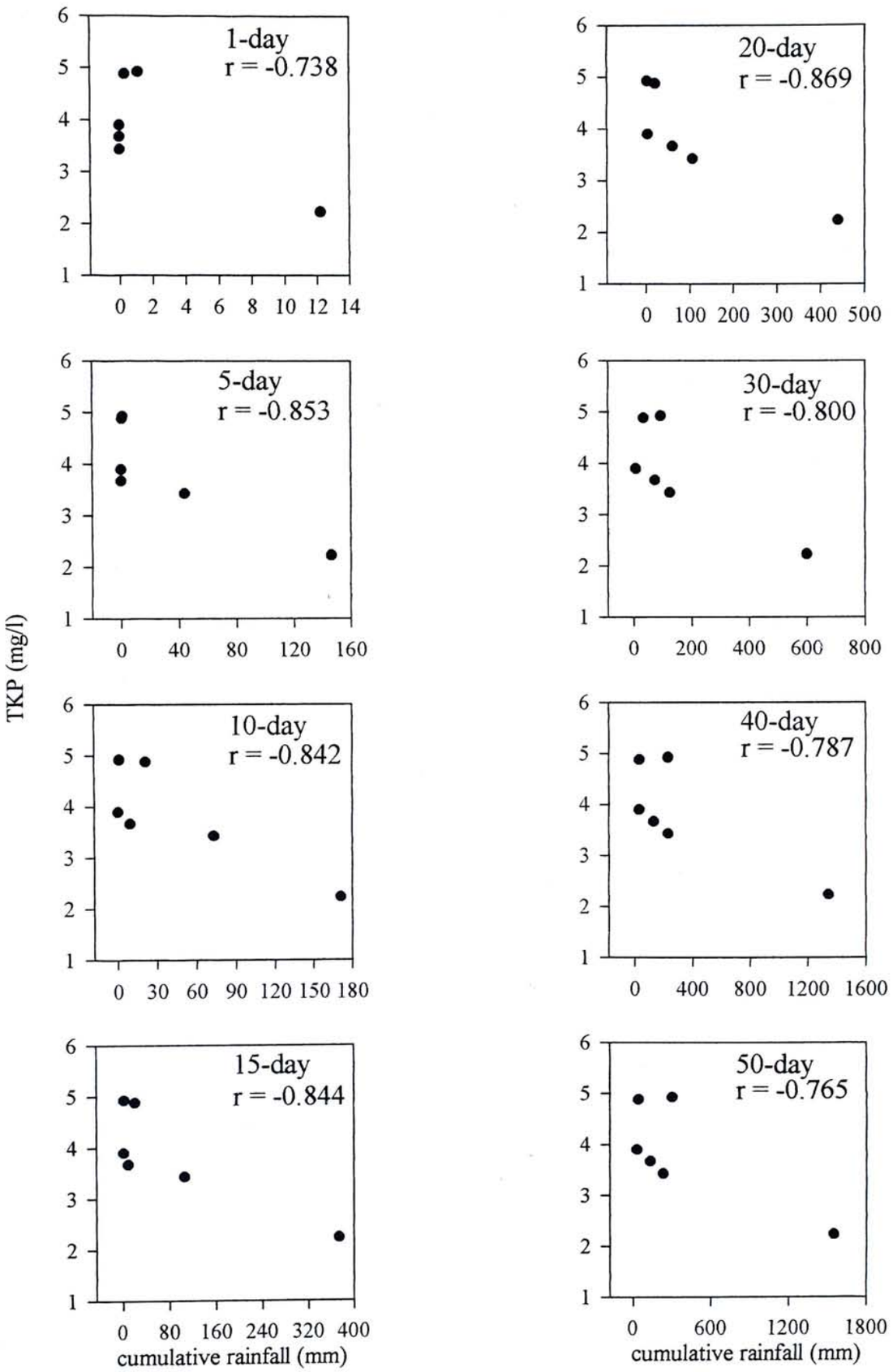


Fig. 2.17 The correlation of total Kjeldahl phosphorus (TKP) of the Pillar Point Valley leachate to 1-day, 5-day, 10-day, 15-day, 20-day, 30-day, 40-day and 50-day cumulative rainfall.

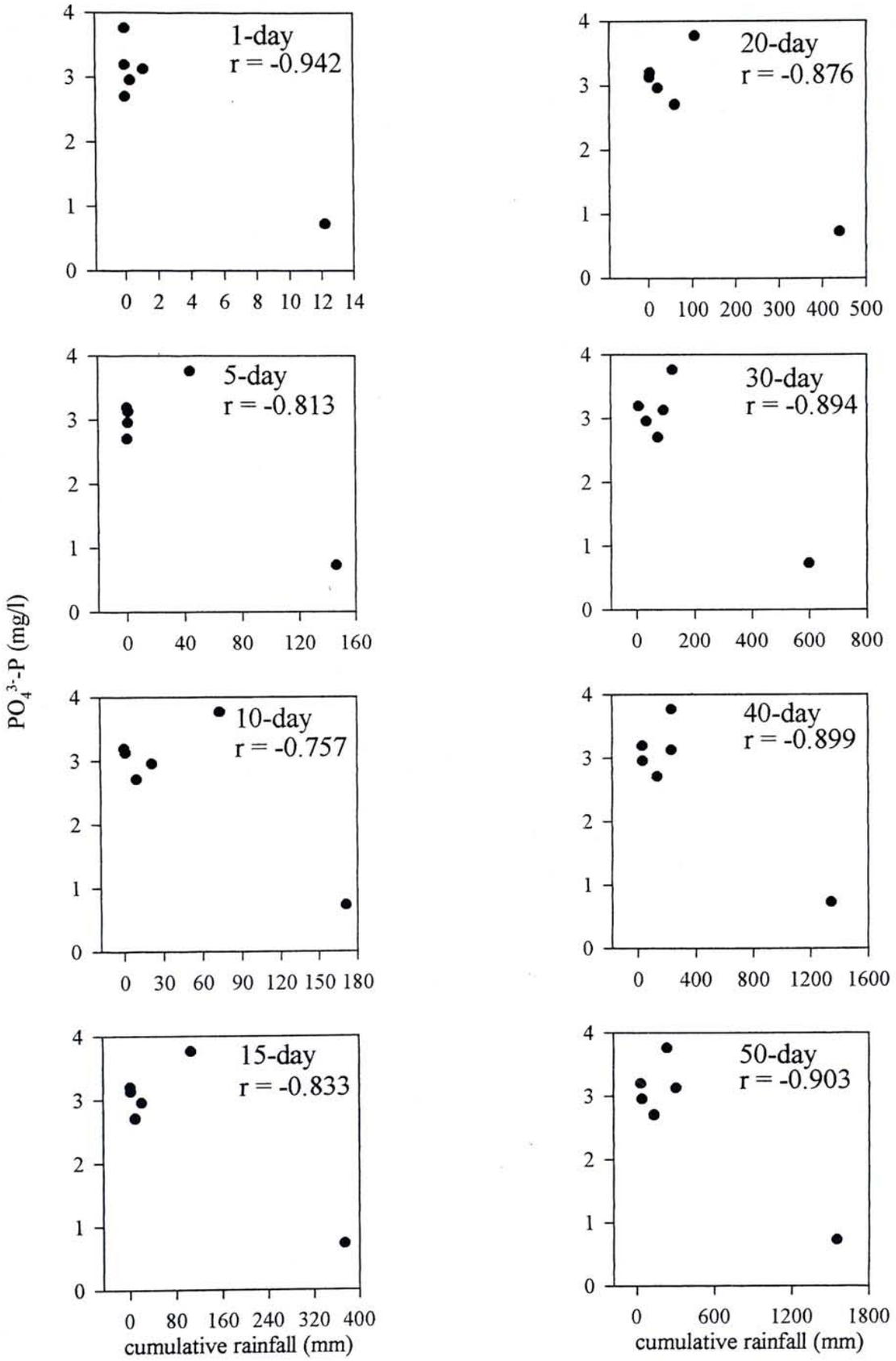


Fig. 2.18 The correlation of orthophosphate-P (PO_4^{3-}-P) of the Ma Yau Tong Central leachate to 1-day, 5-day, 10-day, 15-day, 20-day, 30-day, 40-day and 50-day cumulative rainfall.

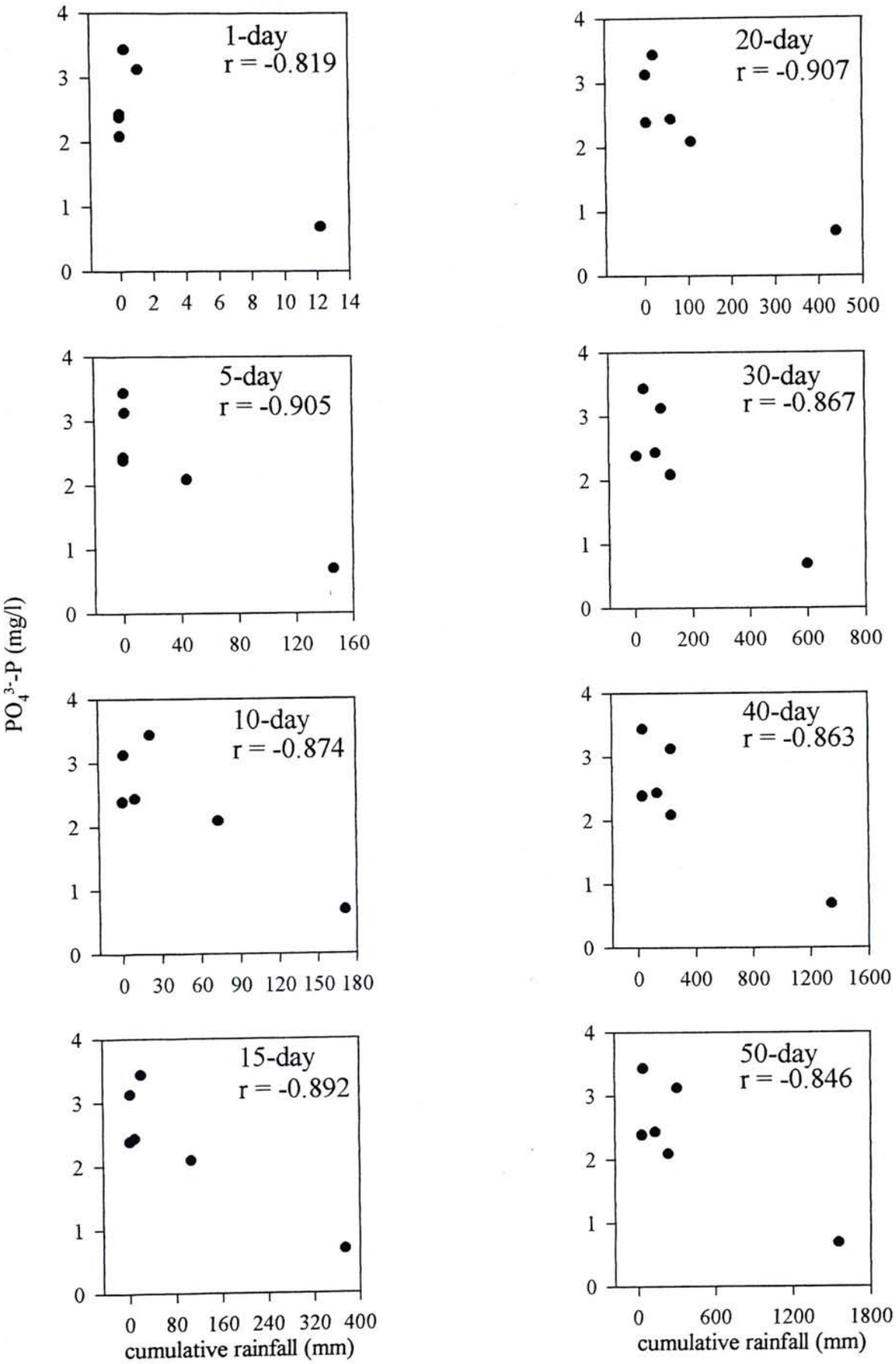


Fig. 2.19 The correlation of orthophosphate-P (PO_4^{3-}-P) of the Pillar Point Valley leachate to 1-day, 5-day, 10-day, 15-day, 20-day, 30-day, 40-day and 50-day cumulative rainfall.

(Table 2.9). For the PPV leachate, TKP concentration was affected more strongly by the percolate which entered recently as significant correlation was found at 3 to 26-day cumulative rainfall and correlation decreased from 27th day and thereafter. For both sites, the degree of correlation of rainfall and orthophosphate was similar. Significant correlation was found almost every day from 1-day to 50-day cumulative rainfall.

Poor correlation was found between heavy metal concentrations and rainfall (Tables 2.10, 2.11 and 2.12). The higher fluctuation of heavy metal concentrations was expected due to precipitation, dissolution, adsorption and complexion which may either retain or mobilize the metals within the landfill.

Different parameters can be classified as follows according to their degrees of correlation with cumulative rainfall.

Good correlation found at both sites ($r > 0.80$, $P < 0.05$)
TS, TKP , PO_4^{3-}-P

Good correlation found at MYT only ($r > 0.80$, $P < 0.05$)
EC, NH_x-N

Intermediate correlation ($0.50 > r > 0.80$)
MYT: pH, COD, TKN, NO_x-N, total/soluble Mn
PPV: DO, total/soluble Zn

Poor correlation ($r < 0.50$)
MYT: DO, salinity, BOD, total/soluble Fe, total/soluble Zn
PPV: pH, EC, salinity, COD, BOD, TKN, NH_x-N, NO_x-N, total/soluble Fe, total/soluble Mn

Parameters such as total solids and phosphorus that are solely affected by short-term dilution have a good correlation with rainfall. They showed good correlation with rainfall regardless of the differences in landfill conditions. For some parameters, concentrations depend on the fill conditions and rainfall. Good correlation with rainfall can only be found in stabilized old landfill. For instance,

good correlation of rainfall and EC or ammonia was found in the stabilized MYT Landfill but not in the younger PPV Landfill. When the fill ages, more parameters will show better correlation with rainfall.

In the operating PPV Landfill, the levels of the principal pollutants, i.e. BOD, COD and ammonia, had poor correlation with cumulative rainfall. This will probably increase the difficulty in estimating leachate quality and the temporal adjustment of the treatment needed by studying rainfall data alone.

The drawback of using rainfall data as an indicator of leachate quality is that the volume of leachate may not be directly proportional to the amount of rainfall. The volume of surface runoff, capacity of wastes to hold water, evaporation, absorption and transpiration all affect water flow into a landfill and hence leachate quality (Baccini *et al.*, 1987; Qasim and Chiang, 1994). However, it is worthwhile to study such a correlation since it provides quick information without the need for determining leachate quality. Knowing of leachate quality in advance allows the modification of treatment process for optimal purification.

2.3.4 Biological composition of leachate

For leachates from both sites, the population of heterotrophic bacteria was higher than that of fungi (Table 2.13). More than 10^4 CFU/mL heterotrophic bacteria were present in leachates from the two landfills. Fungi had a population of less than 200 CFU/mL. Population of the microorganisms was higher in summer period; warmer climate may have promoted the proliferation of the microorganisms.

Microbial populations in the MYT leachate of both the August and February samples were higher than PPV leachate although the MYT leachate had less amount of organic carbon. Some inhibitory factors such as the heat generated by actively

Table 2.13 Populations of total heterotrophic bacteria and fungi in the Ma Yau Tong Central (MYT) and Pillar Point Valley (PPV) leachates collected in August 1994 and February 1995. Values shown are means of 5 replicates.

	Population (CFU/mL)	
	bacteria	fungi
MYT		
Aug 1994	3.58×10^5	160
Feb 1995	7.73×10^4	50
PPV		
Aug 1994	8.70×10^4	178
Feb 1995	6.60×10^4	43

degrading wastes and the presence of toxic compounds, may have suppressed the growth of microorganisms in PPV leachate.

The changing populations of carbohydrate- and protein-utilizing bacteria with season was consistent to the change in substrate concentrations. Concentration of carbohydrates and proteins in the MYT leachate decreased for 63% and 44% respectively from August to February (Table 2.3). Populations of carbohydrate- and protein-utilizing bacteria in the February samples were 83.3% and 22.5% lower that of August respectively (Table 2.14). In the PPV leachate, carbohydrate and protein concentrations were reduced by 84% and 64% respectively while the population of carbohydrate-utilizing bacteria and that of protein-utilizing bacteria were 93.1% and 53.1% lower.

In contrast to the population of total heterotrophic bacteria which was higher in MYT sample, population of carbohydrate- and protein-utilizing bacteria were higher in the PPV leachate. The result is consistent with the concentration of their respective substrates which were higher in the PPV leachate (Table 2.3). Lipid-utilizing bacteria were absent in both leachates (Table 2.14) which agrees with the low lipid concentration in the leachates. Carbohydrate- and protein-utilizing bacteria represented higher proportion of heterotrophic bacteria in the younger landfill.

2.4 CONCLUSIONS

The present study monitored the properties of landfill leachates from a completed site and an operating site. The results showed that the completed landfill was relatively stable in terms of gross organic content with a higher fraction of non-biodegradable compounds as reflected by the BOD:COD ratio. Organic content of

Table 2.14 Populations of carbohydrate-, protein- and lipid-utilizing bacteria in leachates collected from the Ma Yau Tong Central (MYT) Landfill and Pillar Point Valley (PPV) Landfill in August 1994 and February 1995. Values are the means of 5 replicates.

	Population of bacteria (CFU/mL)		
	carbohydrate-utilizing bacteria	protein-utilizing bacteria	lipid-utilizing bacteria
MYT			
Aug 1994	4330	100	ND
Feb 1995	725	77.5	ND
PPV			
Aug 1994	43300	213	ND
Feb 1995	3000	100	ND

ND = not detectable

the operating landfill showed dramatic change. The concentration of biodegradable organics decreased to a low level while that of the non-biodegradable organics was relatively constant. Another principal pollutant in leachates was ammoniacal-nitrogen. Higher ammonia concentration could be found in older site. Options selected for leachate treatment must have high efficiency in the removal of these two major pollutants. Concentrations of phosphorus and heavy metals were low in both leachates. Low heavy metal concentrations eliminate the problem of toxicity to receiving water and biomass of biological treatment plant. However, inadequate phosphorus concentration may limit the growth of microorganisms in biological system for effective treatment.

In Hong Kong, high rainfall and temperature in summer not only promote the stabilization process of landfill, but also lead to seasonal fluctuation in the quality and quantity of leachates. However, the degree of dilution may not be closely related to the rainfall volume. Leachate composition from the older MYT Landfill showed a better correlation with rainfall than that from the PPV Landfill.

Carbohydrates, proteins and lipids accounted for only a small proportion of the organic compounds in the landfill leachates. Their concentrations in the PPV leachate were higher than the MYT leachate. For both sites, concentration of carbohydrates was higher than those of proteins and lipids. The size of bacterial populations using carbohydrates, proteins and lipids followed the same pattern. Heterotrophic bacteria were more abundant than fungi in both leachate. Higher population of heterotrophic bacteria and fungi were found in MYT leachate. However, the indigenous population was much lower than that usually found in municipal sewage which is probably due to the inherent toxicity of landfill leachate. Such a small microbial population may hinder the treatment of the leachate by

biological systems unless an appropriate seeding material is inoculated to ensure adequate microbial biomass for biodegradation.

3 TOXICOLOGICAL ANALYSIS OF LANDFILL LEACHATE

3.1 INTRODUCTION

Chemical characterization is the first step of understanding landfill leachate properties. However, leachate is a complex mixture of organic and inorganic compounds and the list of the compounds present in a leachate is inexhaustive. For a leachate that originates from a variety of wastes, it is difficult, if possible, to know which compound is present and which is not. It is also impractical to determine every compound in a leachate. Moreover, ecotoxicological effect of landfill leachate cannot reflect fully by chemical analysis. Compounds that are responsible for toxicity of a leachate may not be included in the analysis. Interaction of compounds in such a complex matrix as leachate increases the difficulty in predicting toxicity by its chemical composition. Therefore, instead of estimating the ecotoxicological effect of landfill leachate indirectly by chemical analysis, biological tests should be carried out to assess toxicity directly.

In general, two or more toxicity tests with organisms from different trophic levels should be used (Kristensen, 1992). This can avoid wrong estimation of leachate toxicity due to the selection of an inappropriate species. Species from different trophic levels can also provide information on the toxicity effects on organisms that occupy different ecological niches.

Ecotoxicological data for leachate from local landfills are scarce and the suitability of different organisms on testing leachate is also unknown. As there is an increasing concern for environmental problems caused by landfills, the properties of landfill leachate and its ecotoxicological effects have become an important research area. The objectives of this study are (1) to evaluate the suitability of Microtox test,

Chlorella pyrenoidosa, *Moina macrocopa* and *Brachydanio rerio* for testing leachate toxicity, (2) to compare the sensitivities of different toxicity tests to landfill leachates in Hong Kong and (3) to compare the relative toxicity of leachates from a closed landfill and an operating landfill. The chemical properties of a closed and an operating landfill leachates are presented in Chapter 2. Leachates from both landfills had very high concentration of ammonia but concentrations of various toxic heavy metals were very low. In the present study, in addition to parameters measured in the previous chapter, other potential toxic agents, including phenolic compounds, carboxylic acids and total cyanide, were determined.

3.2 Materials and Methods

3.2.1 Leachate collection

Leachates from the Ma Yau Tong Central (MYT) Landfill and Pillar Point Valley (PPV) Landfill were collected in June and December 1994. Samples for chemical analyses were stored in 4.5 L plastic carboys and kept at 4°C upon arrival to laboratory. Samples that could not be analyzed within 24 hours after collection were preserved and stored in ways as recommended by 'standard methods' (American Public Health Association, 1992) within six hours of sampling.

Samples for Microtox test were stored in borosilcate glass bottle at 4°C before experiment according to the Microtox test manual (Microbics Corporation, 1992). Leachate samples for other bioassays were kept in 20 L carboys at 4°C in a cold room prior to laboratory testing. To minimize change of leachate quality before bioassay tests, carboys were closed tightly and no sample was withdrawn before the start of an experiment. The leachate in each carboy was consumed within three days of opening

and leachate remaining in the carboy was discarded.

3.2.2 Chemical analysis

In additional to the parameters measured in Section 2.2.3, concentration of dissolved solids (DS), suspended solids (SS), total alkalinity, carboxylic acids, total cyanide, phenolic compounds, chloride and sulfate were determined for the June and December samples.

Samples for the determination of DS and SS were stored at 4°C according to 'standard methods' (American Public Health Association, 1992) and were analyzed within 6 hours of collection. Samples for the determination of alkalinity and carboxylic acids were stored at 4°C and analyzed by titrimetric method (American Public Health Association, 1992) and distillation method (American Public Health Association, 1992) respectively. Samples for the determination of total cyanide were preserved by adding of sodium hydroxide to pH > 12 and stored at 4°C before analysis by distillation followed by colorimetric method (American Public Health Association, 1992). Samples for phenolic compounds were acidified to pH < 2 by sulfuric acid and stored at 4°C before analysis by 4-aminoantipyrine method using a Lachat QuickChem AE Automated Ion Analyzer. Samples for determination of chloride and sulfate were filtered through Advantec 5C filter paper and stored at 4°C. Chloride and sulfate were measured by thiocyanate method and turbidimetric method respectively using the Lachat QuickChem AE Automated Ion Analyzer.

3.2.3 Biological toxicity testing

The toxicity of leachates was determined using 4 biological systems: including

bacterium (Microtox test®), alga (*Chlorella pyrenoidosa*), crustacean (*Moina macrocopa*) and fish (*Brachydanio rerio*). Microtox test was performed within a week after sample collection. Crustacean and fish toxicity test started within 2 weeks after sample collection. Algal toxicity test started within 4 weeks after sample collection.

3.2.3.1 Microtox test

The Microtox test is a commercial toxicity test using the luminescent bacteria, *Photobacterium phosphoreum*. A Microtox Model 500 Analyzer was used. Toxicity was recorded as the percentage decrease in light output. Basic test protocol was used for determination of EC50 according to Microtox test manual (Microbics Corporation, 1992). The 5-min, 15-min and 30-min EC50 of MYT and PPV leachates were determined.

3.2.3.2 Algal bioassay

The non-mobile unicellular freshwater alga, *Chlorella pyrenoidosa*, was used for toxicity test. Algal culture used for experiment originated from a laboratory culture that was subcultured every week in fresh Bristol's medium (Starr, 1960; Appendix 2) for at least six months. Algal cells for toxicity test was prepared by inoculating the alga in fresh Bristol's medium 4 - 5 days before experiment. Culture was then incubated at 25±2°C, with a light/dark cycle of 14/10 h and light intensity of 2000±30 lux.

Serial dilution of landfill leachates was prepared with distilled water and 80 mL of leachate dilution was placed in each 250 mL conical flask. Neutral pH value of

the MYT and PPV leachate allowed the test to proceed without pH adjustment of leachate dilutions. Solutions were sterilized by autoclaving. Cell density of stock algal culture was determined by a hemocytometer under microscope and adjusted to about 10^6 cell/mL. After the autoclaved leachate dilution had cooled down, 10 mL of 10-fold Bristol's medium and 10 mL of algal inoculum were added to each flask. The initial cell density was 10^5 cell/mL and the final concentrations of leachates were 3, 6, 12, 24 and 48%. Control with Bristol's medium alone was prepared. There were four replicates for control and each leachate concentration. Equal amount of nutrient medium was added to each flask to ensure that any differences in growth were due to the presence of leachate but not the amount of nutrient in the flask. All flasks were incubated in a growth chamber at 25±2°C with a 14/10 h light/dark cycle and a light intensity of 2000±30 lux. No force aeration was employed; flasks were shaken twice a day to facilitate gaseous exchange. Algal growth was monitored on Days 0, 1, 2, 4, 8 and 12th by counting cells in a hemocytometer.

3.2.3.3 Crustacean bioassay

Moina macrocopa was used although water flea of the genus *Daphnia* was more commonly used in toxicity testing. *Moina macrocopa* is a freshwater cladoceran found commonly in small ponds and rice paddies in Southeast Asia. The species is mass cultured by some farmers as a high quality fish food (Wong, 1992). *M. macrocopa* used originated from cultures from the Marine Science Laboratory, The Chinese University of Hong Kong. Individuals for experiment were raised from a single parthenogenetic female and reared in laboratory in 1 L glass beakers at 25±2°C. The beakers were half emptied each month and refilled with tap water that had been

stood overnight. *Chlorella pyrenoidosa* was added every two days to feed the animals.

Newborn neonate less than 24-hour old was used for experiment. They were collected by isolating egg-bearing females from the stock culture 24 hours before experiment. Tests were conducted at 25±2°C with a light/dark cycle of 14/10 h. pH was not adjusted prior to testing as the leachate already has a neutral pH. Range finding test was conducted to identify a suitable range of leachate concentrations for the actual toxicity test. Five neonates were transferred to each 100 mL beaker containing 80 mL of test solution. Leachate was tested at 0 (control), 1, 10 and 100%. Aerated distilled waster was used as dilution water. Dissolved oxygen (DO) concentration in test solution was checked before and after the experiment and to ensure DO levels were higher than 5 mg/L. Although there was no force aeration, the large surface to volume ratio facilitated the gaseous exchange. Mortality was recorded after 24 hours. Immobilization was used as the criteria to define death, i.e. the lack of swimming action and the inability to move except for minor activity of appendages (Atwater *et al.*, 1983; Stephenson *et al.*, 1991). For definitive test of June sample, leachate concentrations tested were 0 (control), 2, 4, 8, 16 and 32%. For definitive test of December sample, leachate concentrations tested were 0 (control), 3, 6, 12, 24 and 48%. Ten neonates were placed in each beaker and four replicates were prepared for each concentration. Test animals were not fed during the experiment. Mortality was recorded after 48 hours. To reduce capture bias, two individuals were transferred to each beaker in sequence (Atwater *et al.*, 1983). After placing equal number of neonates in all beakers, the cycle repeated again until five (for range finding test) and ten (for definitive test) individuals were transferred to each beaker.

3.2.3.4 Fish bioassay

Zebrafish, *Brachydanio rerio*, which is a tropical cypriniform of the family Cyprinidae was used to assess the toxicity of leachate to fish. It is studied extensively because it is easily obtainable, inexpensive and readily maintained (Laale, 1975). Individuals used for the experiment was purchased from a commercial aquarium supply company. Fishes were acclimated to laboratory conditions for two weeks prior to testing. During acclimation period, about 350 - 400 individuals were kept in each of the three 60 cm × 60 cm × 60 cm glass aquaria at 27±2°C, pH 7 - 8 and light/dark cycle 14/10 h. Water used was tap water that had been kept overnight. Fishes were fed twice a day with commercial aquarium fish meal (Momizi tropical for small fish from Yeaster Pet Co., Ltd.). The average size of fishes used for the June and December experiments were 28.5±2.2 mm and 28.8±2.4 mm respectively. Feeding was stopped one day before the start of the experiment. Toxicity tests were conducted in 6 L plastic tanks (25 cm × 15 cm × 17 cm) containing 5 L of diluted leachate. Dilution water was aerated tap water that had been kept overnight. Dilution water alone was used for control. Water in all test tanks was aerated gently by air stone to prevent oxygen deficiency. DO before and after the experiment were checked to ensure the level was greater than 6 mg/L. Sample pH was not adjusted. Temperature was maintained at 27±2°C and a light/dark cycle of 14/10 h was used. Range finding test was conducted to select a suitable range of concentrations for experiment. Two fishes were transferred to tanks containing 0.1, 1, 10 and 100% leachate. Leachate concentrations for definitive test of June and December experiments were 0 (control), 0.25, 0.5, 1, 2 and 4%. Ten fishes were transferred to

each tank and there were four replicates for each leachate concentration. Mortality was observed after 24, 48, 72 and 96 hours. In order to reduce capture bias, the same technique for transferring water flea was employed. Two fishes were transferred to each tank in sequence. After placing equal number of fish in all tanks, the cycle repeated again until ten fishes were placed in each tank.

3.3 Results and Discussion

3.3.1 Chemical properties of leachate

Chemical properties of leachate were listed in Table 3.1. MYT and PPV leachates used for toxicity test had similar pH ranging from 7.15 - 7.42 (Fig. 3.1). Similar pH value were also found in the samples collected in June 1994 to February 1995 (Chapter 2). As it is a common practice to conduct toxicity test at neutral pH (Kristensen, 1992), no pH adjustment was needed for all the toxicity tests.

Effect of solid concentration and color intensity of leachate must be considered as these factors may affect illumination in algal toxicity test (Chu *et al.*, 1994). The effect became more serious in 24% and 48% leachates which were brown in color. For the leachate used in the present study, the soluble fraction represented for more than 96% of total solids (Table 3.1); filtration of the leachate sample could not improve the light transmission.

High oxygen demand of landfill leachate will result in death of test animals. June sample from the PPV Landfill had the highest BOD (Fig. 3.2) and was most likely to result in oxygen depletion problem. In toxicity tests with *M. macrocopa* and *B. rerio*, DO of all leachate dilutions was measured before and after the experiment and was found to be higher than 5 mg/L. Therefore, the death of organisms was not

Table 3.1 Chemical properties of landfill leachates collected from the Ma Yau Tong Central (MYT) and Pillar Point Valley (PPV) Landfill in June and December 1994. Values are the means and standard deviations of four replicates.

	MYT		PPV	
Parameters	June	December	June	December
pH	7.43±0.02	7.28±0.03	7.15±0.04	7.25±0.02
DO (mg/L)	1.95±0.50	3.93±0.21	1.43±0.29	1.44±0.21
EC (μS/cm)	9860±94	8730±35	8240±5	10100±262
Salinity ($^{o}/_{oo}$)	7.25±0.50	6.00±0.00	7.00±0.00	8.00±0.00
Total alkalinity (mg/L)	7740±1020	5990±68	6150±137	6360±46
TS (mg/L)	2350±44	1920±9	2910±74	3300±29
SS (mg/L)	26.7±10.3	22.2±3.64	92.3±39.5	19.1±7.3
DS (mg/L)	2320±79	1893±22	2820±78	3273±15
COD (mg/L)	730±75	547±31	643±107	807±21
BOD (mg/L)	72.8±11.3	31.1±8.0	259±45	47.9±3.3
Carboxylic acids (mg/L)	16.3±1.3	34.4±0.7	114±8	127±1
TKN (mg/L)	1130±15	815±14	684±85	731±10
NH_x-N (mg/L)	850±265	803±12	620±89	649±48
NO_x-N (mg/L)	0.76±0.11	0.75±0.07	0.11±0.01	0.12±0.02
TKP (mg/L)	5.45±0.27	4.51±0.21	3.43±0.83	3.67±0.32
PO_4^{3-}-P (mg/L)	3.77±0.10	2.71±0.14	2.09±0.27	2.44±0.07
SO_4^{2-}-S (mg/L)	57.7±10	63.5±1.8	43.0±2.87	83.7±2.7
Phenolic compounds (mg/L)	ND	ND	ND	ND
Cl^-(mg/L)	529±25	420±6	634±22	790±8
CN^- (mg/L)	ND	ND	ND	ND
Total metal (mg/L)				
Cd	ND	ND	ND	ND
Cr	ND	ND	ND	ND
Cu	ND	ND	ND	ND
Fe	12.3±0.6	13.5±1.4	43.8±12.3	16.3±4.3
Mn	0.82±0.55	1.02±0.03	8.22±0.46	3.10±0.70
Ni	ND	ND	ND	ND
Pb	ND	ND	ND	ND
Zn	0.10±0.05	0.15±0.02	0.05±0.00	0.15±0.01
Soluble metal (mg/L)				
Cd	ND	ND	ND	ND
Cr	ND	ND	ND	ND
Cu	ND	ND	ND	ND
Fe	11.5±0.8	9.21±1.13	19.8±2.6	11.1±1.1
Mn	0.42±0.05	0.96±0.13	6.91±0.34	3.17±0.38
Ni	ND	ND	ND	ND
Pb	ND	ND	ND	ND
Zn	0.07±0.02	0.11±0.03	0.03±0.01	0.15±0.02

ND = not detectable

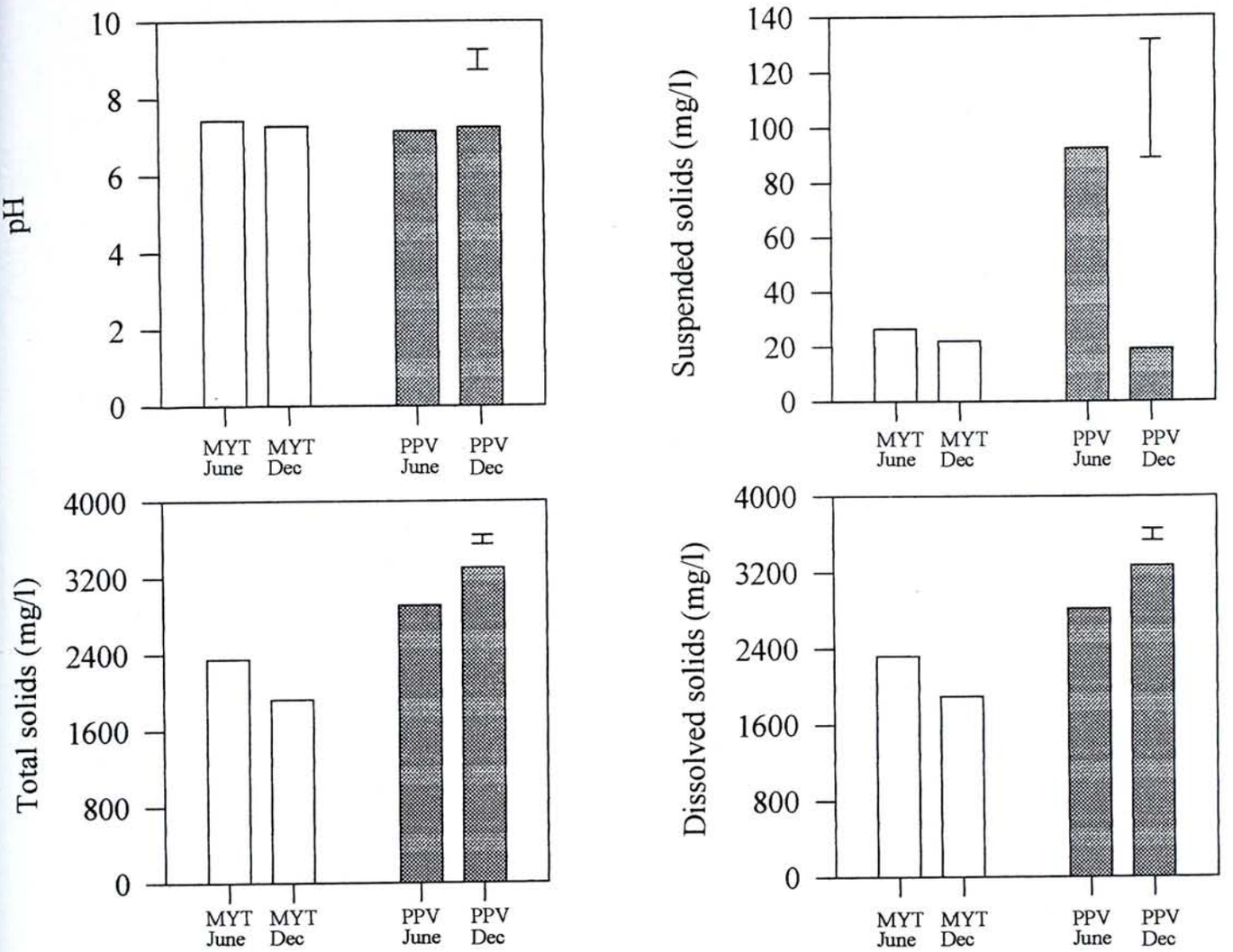


Fig. 3.1 pH, concentrations of total solids, suspended solids and dissolved solids of the MYT (empty bar) and PPV leachates (solid bar) collected in June and December 1994. Values are the means of 4 replicates. Vertical bars denote LSD by Tukey's Honestly Significant Difference Test at $P = 0.05$.

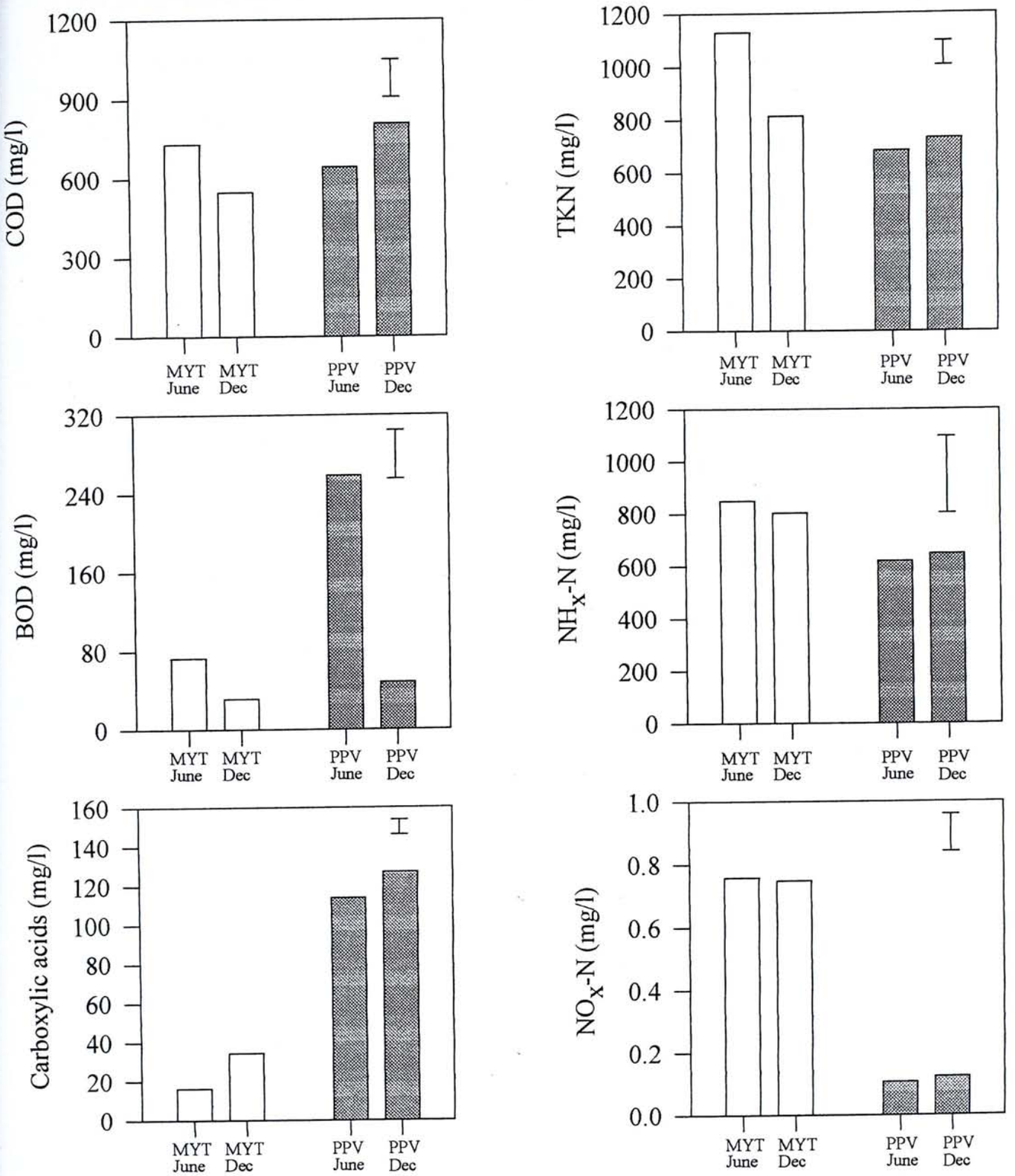


Fig. 3.2 COD, BOD, and concentrations of carboxylic acids, total Kjeldahl nitrogen, ammoniacal-N and oxidized-N of the MYT (empty bar) and PPV leachates (solid bar) collected in June and December 1994. Values are the means of 4 replicates. Vertical bars denote LSD by Tukey's Honestly Significant Difference Test at $P = 0.05$.

due to oxygen depletion.

Concentrations of carboxylic acids, ammonia, phenolic compounds, cyanide, chloride and metals were determined in order to assess their contributions to leachate toxicity. Carboxylic acid concentration of the MYT leachate was significantly lower than the PPV leachate (Fig. 3.2). For MYT leachate, only 16.3 mg/L was found in June sample and 34.4 mg/L was found in December sample. In the operating PPV Landfill, concentrations of carboxylic acids in June and December samples were 114 mg/L and 127 mg/L respectively (Table 3.1). Carboxylic acid concentrations in the MYT and PPV leachates were considered to be low when compared with acetogenic leachate which has fatty acid concentration of several thousand mg/L (Harmsen, 1983).

Concentration of ammonia in leachate was high. Ammonia was the major pollutant and toxic agent in leachate. Ammoniacal-N concentrations of the MYT-June, MYT-December, PPV-June and PPV-December samples were 850, 803, 620 and 649 mg/L respectively (Table 3.1). These were equivalent to 12.8, 12.1, 9.34 and 9.78 mg/L of free ammonia respectively at 25°C and the pH of the corresponding leachate according to Eq. 4.7. Acute toxicity effect for salmonid and non-salmonid fish species was found between 0.1 and 10 mg/L unionized ammonia (Environmental Protection Agency, 1989). Thus, both MYT and PPV leachates may cause acute toxic effect to fish species.

Concentrations of other hazardous compounds such as phenolic compounds and cyanide were too low to measure. For heavy metals, only iron, manganese and zinc were present at significant levels in both leachates. Their concentrations were higher in the PPV leachate than in the MYT leachate (Fig. 3.3).

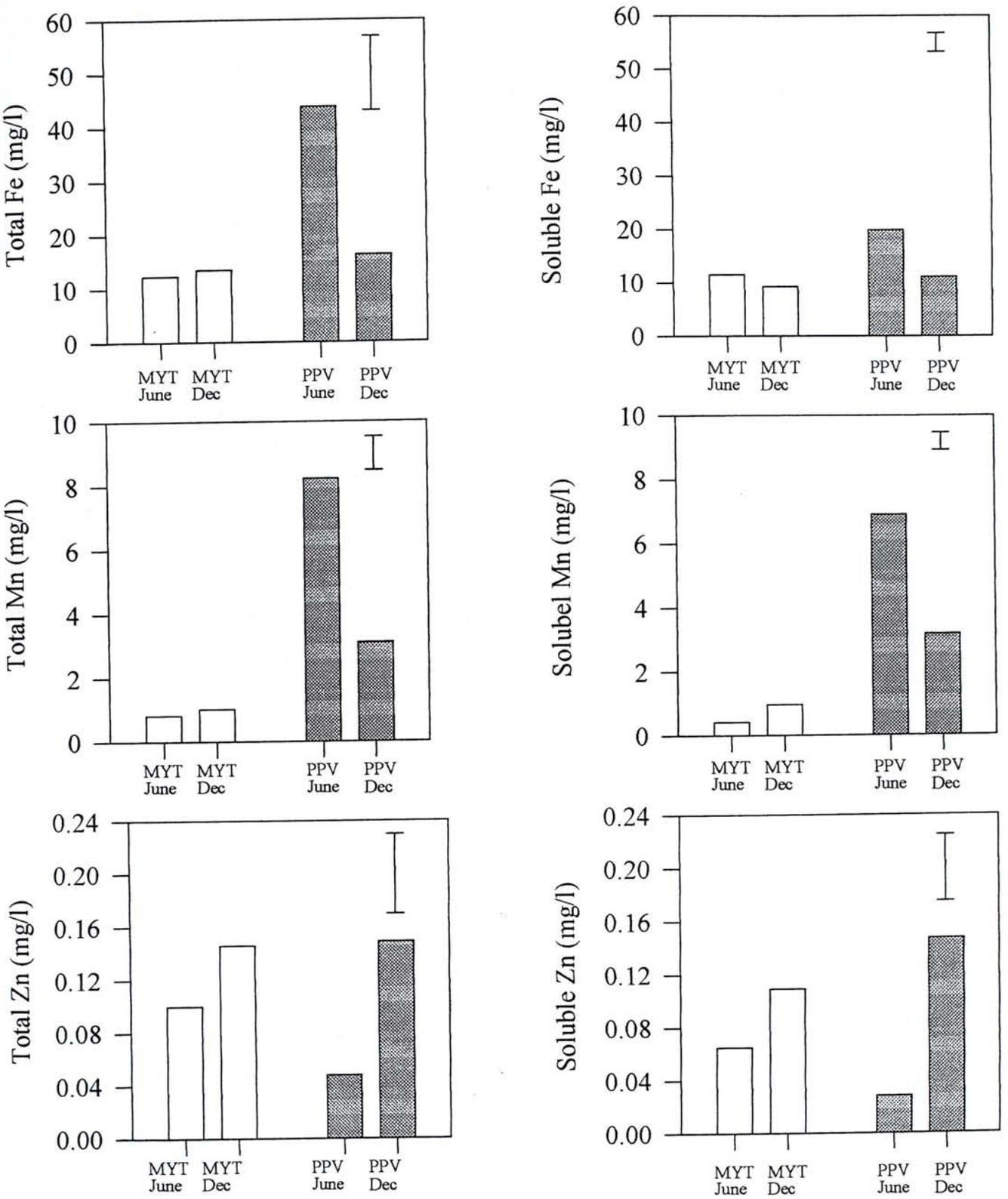


Fig. 3.3 Concentrations of total and soluble iron, manganese and zinc of the MYT (empty bar) and PPV leachates (solid bar) collected in June and December 1994. Values are the means of 4 replicates. Vertical bars denote LSD by Tukey's Honestly Significant Difference Test at $P = 0.05$.

3.3.2 Microtox test

Results of the June and December samples from the PPV Landfill were conflicting (Table 3.2). EC50 of June sample decreased with exposure time, while that of December sample increased with time. 5-min EC50 of the PPV June sample was higher than 100% of leachate. EC50 decreased gradually to 43.4% for 15 min test and further decreased to 28.5% for 30 min test. The decrease of EC50 with time indicates the toxic agents was/were slow acting. On the other hand, EC50 value of December sample increased with exposure time. The light emission of the bacterium seemed to recover when exposure time increased. Differences in the properties of June and December PPV leachates might explain conflicting results. According to Section 2.3.1, BOD of PPV leachate decreased during the sampling period, from 259 mg/L in June to 47.9 mg/L in December. Microtox test is highly sensitive to pure organics but is less sensitive to most inorganics (Munkittrick *et al.*, 1991). Although individual organic compounds were not identified in the present study, dramatic change in BOD and consequently concentration of organics in the PPV leachate might explain the differences between June and December results. However, due to the change of EC50 value of PPV sample along with time, it was difficult to tell whether MYT or PPV samples were more toxic.

While ammonia is a major pollutant in methanogenic leachate, Microtox test is a poor indicator of ammonia toxicity. It required 3607 mg/L ammonia to induce 50% inhibition of light emission (Table 3.3). In contrast, the 48-h LC50 for *Daphnia magna* and 96-h LC50 for rainbow trout were 129 and 62 mg/L ammonia respectively. Ammonia concentrations in the MYT and PPV leachates were 803 -

Table 3.2 5-min, 15-min and 30-min EC50 of leachates detected by Microtox test, basic protocol. The leachates were collected from the Ma Yau Tong Central and Pillar Point Valley Landfills in June and December of 1994.

Duration (min)	EC50 (%, v/v)	
	Ma Yau Tong	Pillar Point Valley
June		
5-min	55.1	>100
15-min	61.9	43.4
30-min	62.5	28.5
December		
5-min	48.1	37.2
15-min	55.1	35.1
30-min	49.2	63.5

Table 3.3 Comparison of the relative toxicity of pure organic and inorganic chemicals to Microtox test, *Daphnia*, rainbow trout and fathead minnow (Munkittrick *et al.*, 1991).

Chemicals	Microtox test			*D. magna*	Rainbow trout	Fathead minnow
	5 min EC50 (mg/L)	15 min EC50 (mg/L)	30 min EC50 (mg/L)	48-h LC50 (mg/L)	96-h LC50 (mg/L)	96-h LC50 (mg/L)
Organic						
phenols	22.0	-	-	32.0	9.9	-
	21-41	-	-	10-23	-	23-24
	22.0-40.2	34.0	-	7.0-88	5.0-11.6	24.0-67.5
	25	-	-	-	8.9	3.5
chlorophenol						
2	0.54	0.51	0.58	<0.77	-	-
4	1.18	1.15	1.19	1.17	-	-
2,4	1.54	1.51	1.47	1.21	-	-
2,4,5	2.22	2.21	2.19	1.71	-	-
2,4,6	1.52	1.38	1.41	1.10	-	-
2,3,4,6	2.09	2.20	2.26	<2.37	-	-
2,3,5,6	1.92	1.96	2.02	1.97	-	-
2,3,4,5,6	2.46	2.64	2.71	2.25	-	-
3,5	3.0-4.5	-	-	1.7-4.0	-	8.3-9.7
pentachloro	0.08-0.15	-	-	0.14-0.28	-	0.2-0.5
-phenol	-	1.0	-	0.1	-	0.3
Inorganic						
total ammonia	3607	-	-	129	62	-
unionized ammonia	1.5	-	-	0.8	1.4	-
cyanide	13.3	-	-	6.1	0.15	-
	2.8-3.5	-	-	0.08-0.1	-	0.1-0.2
cadmium	70-90	10	-	0.02-0.16	-	0.01-0.14
	-	-	7-60	-	-	55
	-	-	14	-	-	2.2
	106	25	-	0.041	-	-
chromium	70-100	13	-	0.10-0.13	-	12-53
	-	-	42-58	-	-	31
cobalt	135-177	16	-	4.7-13	-	50-70
copper	7.4	-	-	0.02	0.25	-
	4-20	-	-	0.02	0.25	-
	-	-	0.5-2.0	-	-	78
	1.2	0.42	0.24	0.064	-	-
zinc	49	-	-	5.1	2.2	-
	2-14	-	-	1.0-1.2	-	0.5-1.7
	-	1.4-8	-	-	-	66
	12	1.6	0.7	0.54	-	-

850 mg/L and 620 - 649 mg/L respectively which were much lower than the reported EC50 value.

In addition to ammonia, Microtox test also showed poorer sensitivities to inorganic compounds such as cyanide and heavy metals when compared with other biological toxicity methods (Table 3.3). Concentrations of cyanide, heavy metals, phenolic compounds in the MYT and PPV leachates (Table 3.1) were below the stated toxic levels (Table 3.3).

Salts and organic compounds in Microtox test reagents reduce the amount of free ion or ligands present in the leachate. The presence of high concentrations of calcium and nitrogen nutrient in leachate could stimulate the light output of the fluorescent bacterium (Kross and Cherryholmes, 1994) and underestimated the toxicity of leachate.

Although Microtox test is relatively insensitive to ammonia, it is a useful tool for determining toxicity caused by chemicals other than ammonia. When aquatic animals such as fish is used, ammonia toxicity may overwhelm the toxicity effects of other chemicals.

3.3.3 Algal bioassay

Inhibition of algal growth was only observed at 24% and 48% leachate for all four samples (Figs. 3.4 - 3.7). Algal growth was better in lower leachate concentrations (3% and 6%) than in the control (Bristol's medium). Algal densities of 3%, 6% and 12% leachate from the MYT Landfill were higher than that of the control after 8 days (Figs. 3.4 and 3.5). For the PPV leachate, cell densities at 3%, 6% and 12% leachate were higher than that of the control after 2 days (Figs. 3.6 and

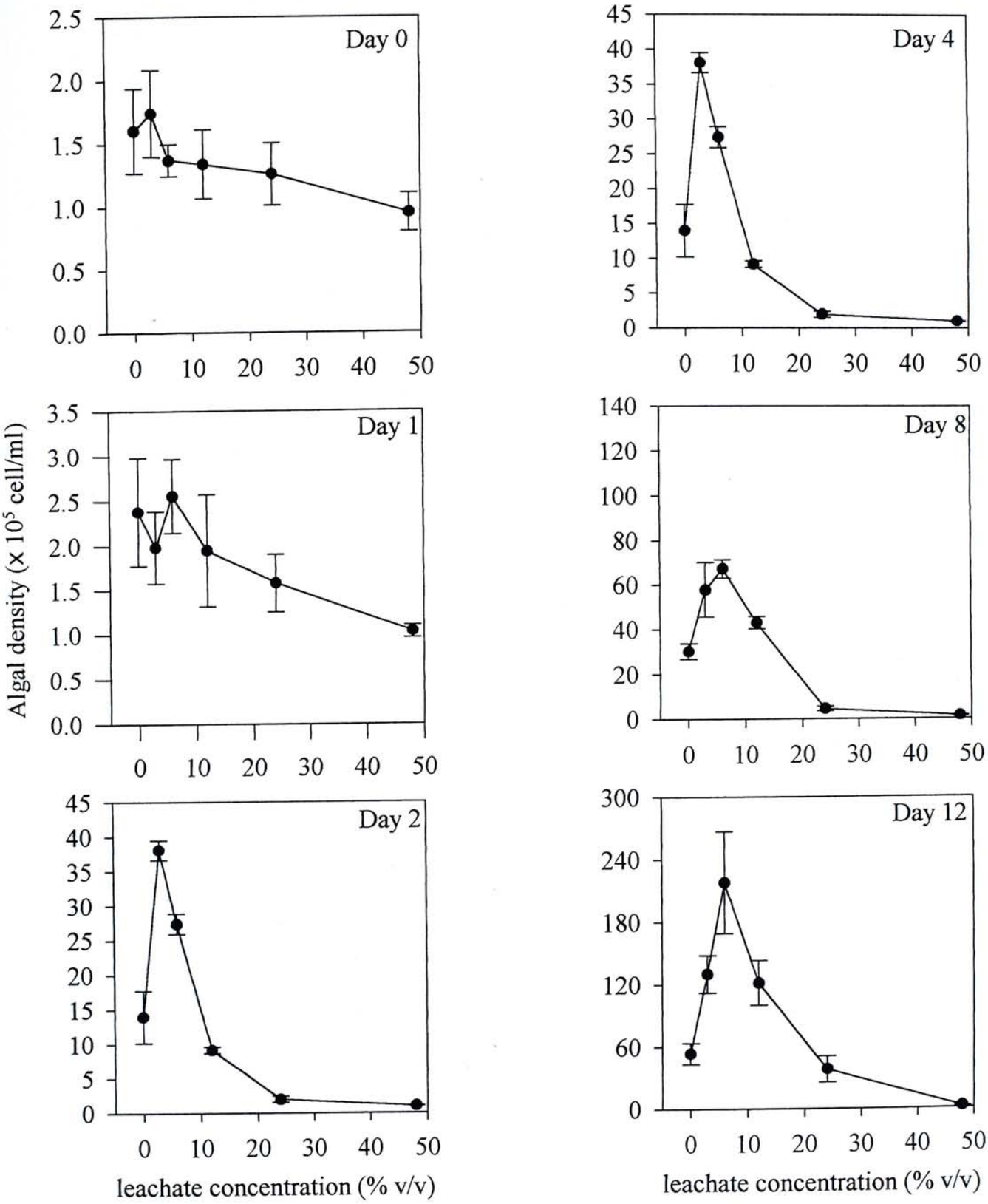


Fig. 3.4 Cell density of *Chlorella pyrenoidosa* at 0% (control), 3%, 6%, 12%, 24% and 48% of leachate. Leachate was collected from the Ma Yau Tong Central (MYT) Landfill in June 1994. Experiments lasted for 12 days. Values are the means of 4 replicates and vertical bars denote standard deviation.

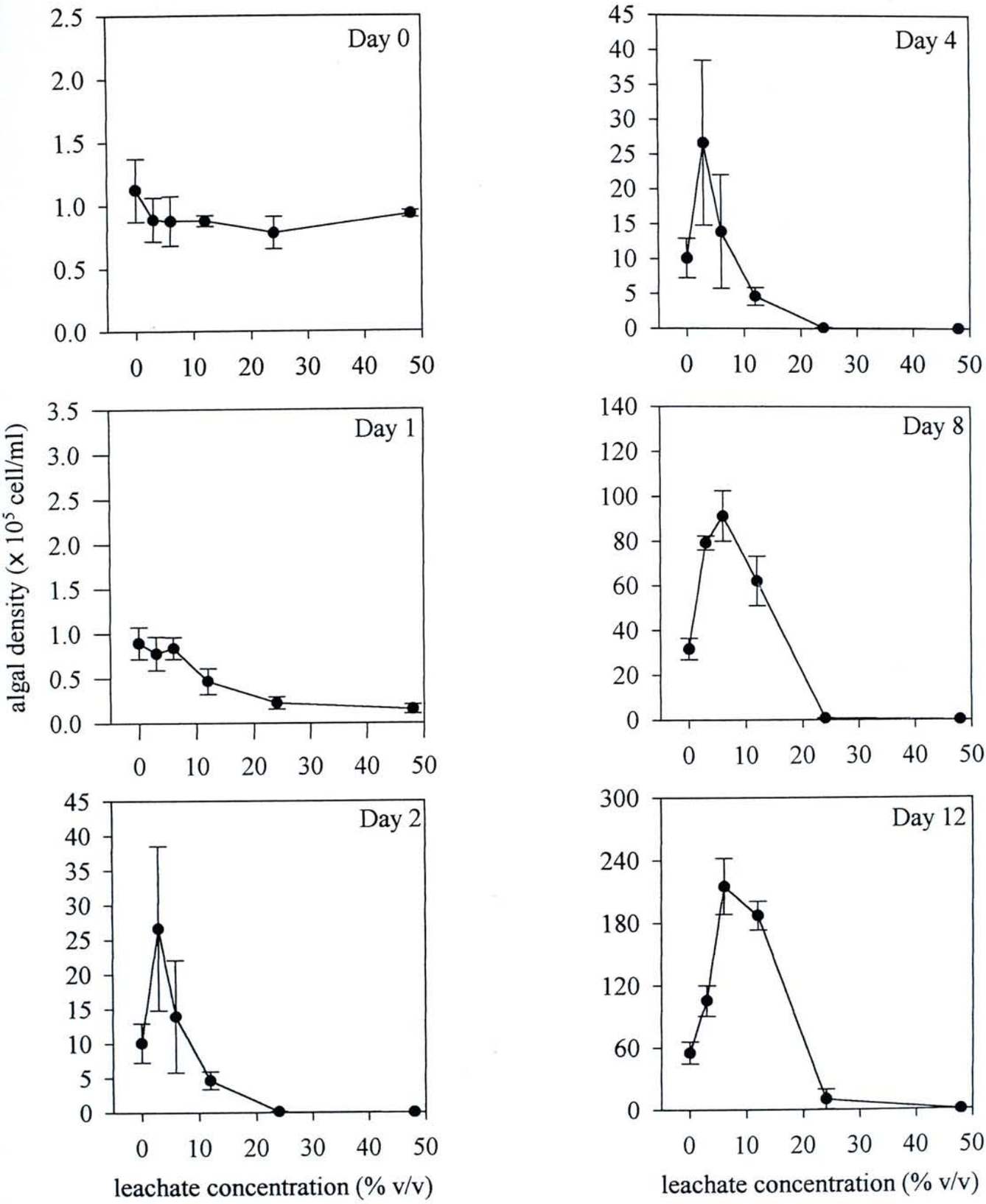


Fig. 3.5 Cell density of *Chlorella pyrenoidosa* at 0% (control), 3%, 6%, 12%, 24% and 48% of leachate. Leachate was collected from the Ma Yau Tong Central (MYT) Landfill in December 1994. Experiment lasted for 12 days. Values are the means of 4 replicates and vertical bars denote standard deviation.

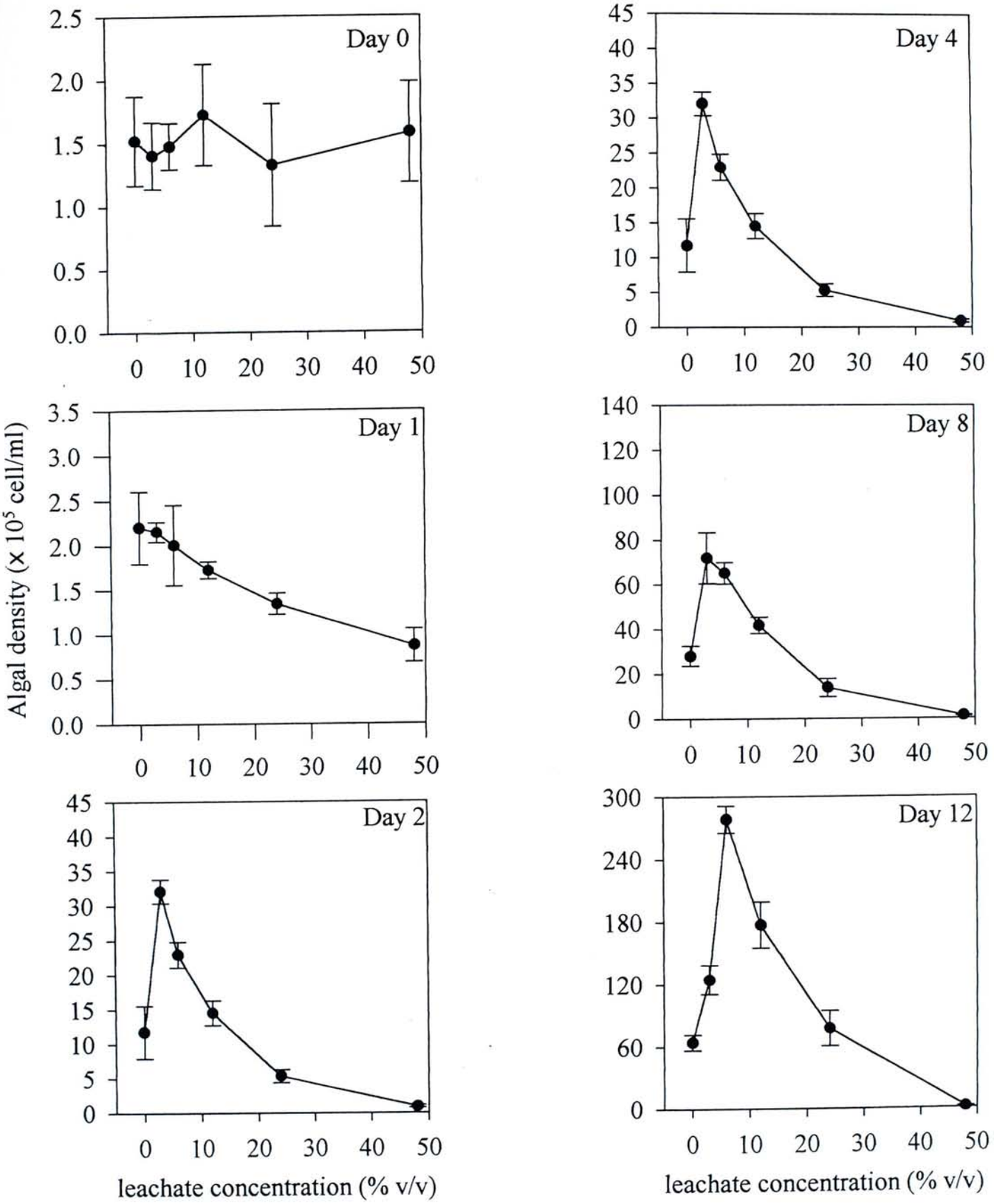


Fig. 3.6 Cell density of *Chlorella pyrenoidosa* at 0% (control), 3%, 6%, 12%, 24% and 48% of leachate. Leachate was collected from the Pillar Point Valley (PPV) Landfill in June 94. Experiment lasted for 12 days. Values are the mean of 4 replicates and vertical bars denote standard deviation.

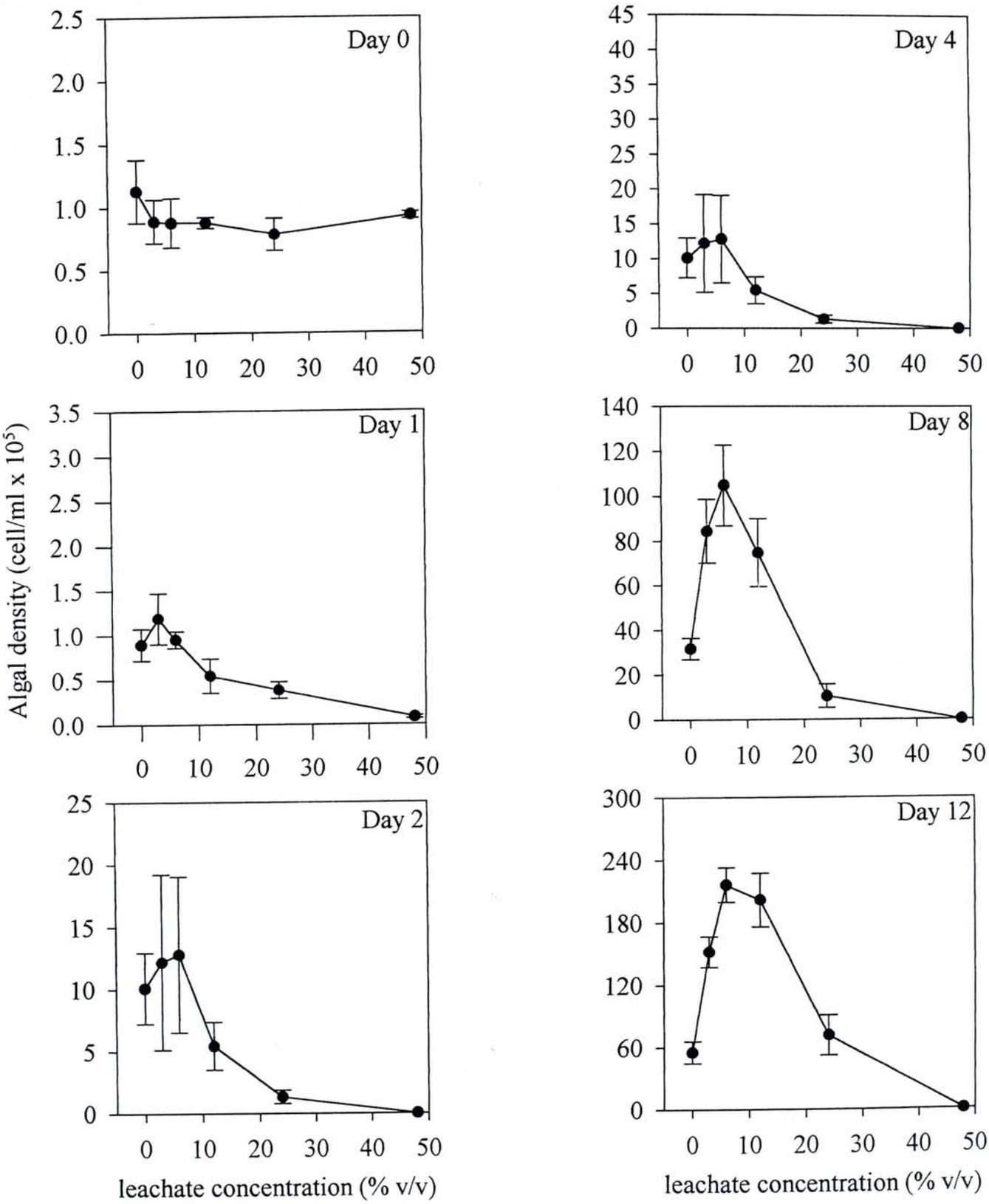


Fig. 3.7 Cell density of *Chlorella pyrenoidosa* at 0% (control), 3%, 6%, 12%, 24% and 48% of leachate. Leachate was collected from the Pillar Point Valley (PPV) Landfill in December 1994. Experiment lasted for 12 days. Values are the means of 4 replicates and vertical bars denote standard deviation.

3.7). Cell density declined to very low levels at leachate concentration greater than 24%.

It had been reported that 31.5 mg/L of ammoniacal-N inhibits the growth of *C. pyrenoidosa* (Wong *et al.*, 1984). Although autoclaving caused a 15 - 30% loss of ammonia (Table 3.4), 6% of the June samples and 12% of the December samples should still have ammonia at levels inhibitory to the growth of *C. pyrenoidosa*. Surprisingly, instead of inhibition, growth stimulation was found at these concentrations. This may be account for by a decrease in ammonia concentration during experiment, acclimation of algae to leachate and nutrients provided by the leachate. Photosynthesis increased the pH of the medium to alkaline and facilitated the loss of ammonia as gas. Ammonia was also utilized by algae. These two factors resulted in a decrease in ammonia to below the toxic level as experiment was progressing. Prolonged experiment allowed the alga to acclimate to leachate at later generations. Nutrients provided by leachate might also stimulate cell growth. *C. pyrenoidosa* utilizes ammonia preferentially as they do not produce active nitrate reductase in stationary culture (Tam and Wong, 1990). When *C. pyrenoidosa* was cultured in medium containing both ammonia and nitrate, decrease in nitrate concentration occurred only after ammonia concentration had fallen below a certain threshold. Bristol's medium (Appendix 2) contained nitrate as the only nitrogen source. *C. pyrenoidosa* used ammonia originated from leachate preferentially instead of nitrate provided by medium. Use of nutrient media containing ammonia cannot solve the present problem. Unless toxic level of ammonia is reached, *Chlorella* will use ammonia as a source of nitrogen. Addition of more ammonia would boost the growth of *Chlorella* as long as ammonia concentration was below toxic level.

Table 3.4 Ammoniacal-N concentration of leachate dilution before and after autoclaving at 121°C for 20 min. Leachates were collected from the Ma Yau Tong Central (MYT) and Pillar Point Valley (PPV) Landfills in June and December 1994. Values are the means of 4 replicates.

Leachate conc. (v/v)	Ammoniacal-N (mg/L)							
	MYT June		PPV June		MYT Dec		PPV Dec	
	Before	After	Before	After	Before	After	Before	After
3%	25.5	21.4	24.1	17.3	18.6	15.5	19.5	12.8
6%	51.0	42.2	48.2	37.8	37.2	30.6	38.9	22.3
12%	102	82.3	96.4	97.8	74.4	64.3	77.9	55.6
24%	204	172	193	148	149	121	156	102
48%	408	338	385	270	298	218	312	209

3.3.4 Crustacean bioassay

Results *M. macrocopa* toxicity test are showed in Fig. 3.8. The 48-h LC50 was calculated by probit method (Petrocelli and Rand, 1985). June samples were more toxic than those collected in December from both the MYT and PPV leachates (Table 3.5). Samples collected from the MYT Landfill were more toxic than those from the PPV Landfill in both dates. None of the monitored parameter show a clear trend in concentration similar to that of toxicity. The observed effect was likely due to a combined effect of several toxic components.

Toxic effect of heavy metals to *M. macrocopa* has been studied extensively. Concentrations of heavy metals in the leachates dilutions (Table 3.1) were much lower than the 48-h LC50 reported for *Moina* (Table 3.6). Heavy metals were probably not the major causatic factor for the observed toxicity effect of landfill leachate.

Water flea seemed to be more convenient test organisms for routine toxicity test than both algal and fish. In addition to its sensitivity to leachate, *M. macrocopa* is easy to maintain. Genetically homogenous populations can be obtained from a single parthenogenetic female. Variation due to genetic variation can be minimized and this is an important advantage over other test organisms. Equipment and technique required for experiments are simple and inexpensive.

3.3.5 Fish bioassay

All the samples showed a 100% survival in 2% leachate throughout 96 hours of exposure (Tables 3.7 and 3.8) but 100% death was observed in 4% and 8% leachate in all except the PPV June sample after just 24 hours. For 4% leachate of the

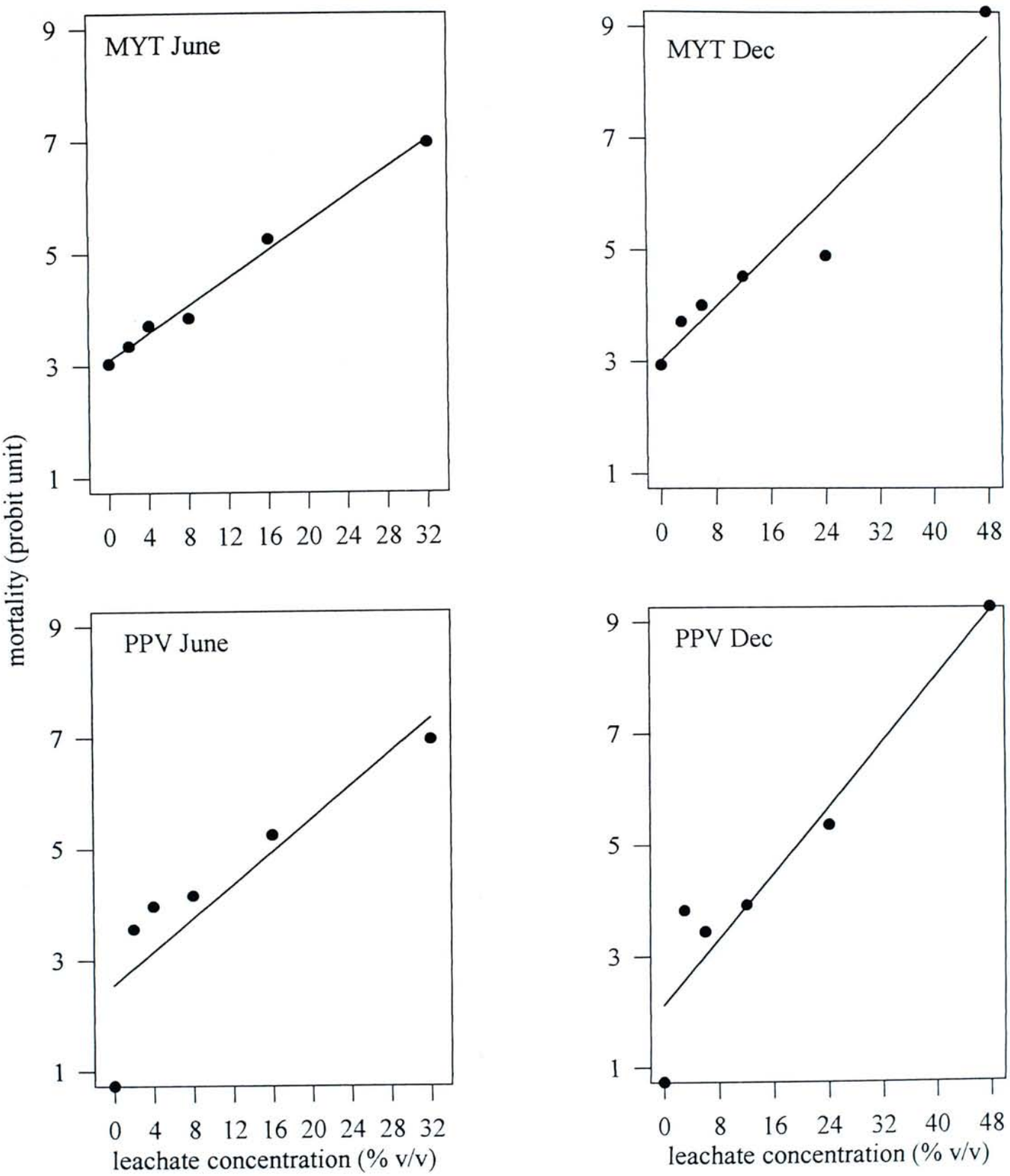


Fig. 3.8 Mortality of *Moina macrocopa* after exposed to leachate for 48 hours. Leachates were collected from Ma Yau Tong Central (MYT) Landfill and Pillar Point Valley Landfill in June and December 1994. Values are the means of 4 replicates.

Table 3.5 48-h LC50 of *Moina macrocopa* exposed to landfill leachates collected from the Ma Yau Tong Central (MYT) and Pillar Point Valley (PPV) Landfills in June and December 1994.

	48-h LC50 (%, v/v)	
	MYT	PPV
June	15.5	16.4
December	16.3	19.4

Table 3.6 48-h LC50 of heavy metals for *Moina macrocopa* and *Moina irrasa.*

Metals (mg/L)					
Cd	Cr	Cu	Ni	Zn	Reference
Moina macrocopa					
-	0.36	0.08	6.48	1.17	Wong, 1992
0.07	-	-	-	-	Hatakeyama and Yasuno, 1981
Moina irrasa					
0.02913-0.05516	-	0.0063-0.01261	-	0.07746-0.20531	Zou and Bu, 1994

Table 3.7 Mortality of *Brachydanio rerio* under different concentrations of the MYT and PPV leachates collected in June 1994.

Exposure duration	Mortality (%)					
	Control	0.5%	1%	2%	4%	8%
24 h						
MYT	0	0	0	0	100±0	100±0
PPV	0	0	0	0	52.8±20.6	100±0
48 h						
MYT	0	0	0	0	100±0	100±0
PPV	0	0	0	0	77.5±26.3	100±0
72 h						
MYT	0	0	0	0	100±0	100±0
PPV	0	0	0	0	82.5±20.6	100±0
96 h						
MYT	0	0	0	0	100±0	100±0
PPV	0	0	0	0	82.5±20.6	100±0

Table 3.8 Mortality of *Brachydanio rerio* under different concentrations of the MYT and PPV leachates collected in December 1994.

Exposure duration	Mortality (%)					
	Control	0.5%	1%	2%	4%	8%
24 h						
MYT	0	0	0	0	100±0	100±0
PPV	0	0	0	0	100±0	100±0
48 h						
MYT	0	0	0	0	100±0	100±0
PPV	0	0	0	0	100±0	100±0
72 h						
MYT	0	0	0	0	100±0	100±0
PPV	0	0	0	0	100±0	100±0
96 h						
MYT	0	0	0	0	100±0	100±0
PPV	0	0	0	0	100±0	100±0

PPV June sample, mortality after 24 to 96 hours exposure ranged from 52.8 to 82.5%. Total death was found in 8% leachate.

LC50 should be between 2% to 8% leachate for the PPV June sample and 2% to 4% leachate for other samples. Because DO of diluted leachate was higher than 6 mg/L, it is not likely that death of fish was due to oxygen depletion. Zebrafish was very sensitive to component(s) in the MYT and PPV leachates.

Eggs and larvae of zebrafish were extremely susceptible to metals. Copper or lead concentration of 72 μg/L could reduce hatching for about 40% (Ozoh, 1979). Hatching of egg was not affected when concentrations of copper, mercury, lead, nickel were as low as 0.05, 10, 20 and 40 μg/L respectively; the survival of larvae was not affected if concentrations of the metals were 0.25, 1.2, 30, and 80 μg/L respectively (Dave and Xiu, 1991). However, the response of adult fish (that was used in the present study) is rarely reported, and data presented here was not enough to pinpoint which component(s) is/are responsible for death of zebrafish. Zebrafish is a very sensitive subject to detect the presence of leachate in receiving water. The disadvantage of this bioassay is that it is easy to underestimate or overestimate if the critical concentration is missed.

If zebrafish is used as the test subject for detecting leachate toxicity, a more precise range finding test must be performed to identify the range of test concentrations.

3.4 CONCLUSIONS

The suitability and sensitivity of Microtox test, *Chlorella pyrenoidosa, Moina macrocopa* and *Brachydanio rerio* for assessing leachates toxicity were studied. The

range of sensitivity was *Brachydanio rerio* > *Moina macrocopa* > Microtox test > *Chlorella pyrenoidosa* (Table 3.9).

Although Microtox test showed low sensitivity to the MYT and PPV leachates, it is a very useful tool for determining leachate toxicity caused by compounds other than ammonia. As fish and crustaceans were more sensitive to ammonia and the leachate studied contained very high concentrations of ammonia, it is likely that toxic effects of ammonia might mask the effects of other chemicals. The low sensitivity of Microtox test to ammonia can provide a useful tool to overcome this problem.

Among the four test organisms, *C. pyrenoidosa* was the least suitable. Due to the complex nature of its chemical composition, leachate can provide a variety of macro- and micronutrients to algae. Indeed, growth of *C. pyrenoidosa* was stimulated by landfill leachate at low concentrations.

Zebrafish showed the highest sensitivity to leachate toxicity. Mortality of 100% was found at 4 - 8% of leachate. A precise range finding test must be done to find the suitable range of leachate concentrations for testing.

M. macrocopa appeared to be the best organism for leachate testing. It was fairly sensitive and gave a linear relationship between mortality and leachate concentration. In addition, the test procedure was simpler when compared to those for fish and algae.

According to the results of water flea bioassay, MYT samples were more toxic than PPV samples and the June samples were more toxic than their corresponding December samples. Although no LC50 could be calculated from the toxicity test using fish, MYT June sample was considered more toxic than PPV June sample as

Table 3.9 Results of four bioassays on testing landfill leachates collected from the Ma Yau Tong Central (MYT) and Pillar Point Valley (PPV) Landfills in June and December 1994.

Bioassay	Effect assessed	Result
Microtox test (*Photobacterium phosphoreum)*	Light emission	5, 15 and 30 min EC50: MYT June sample: 55.1-62.5% MYT Dec sample: 48.1-55.1% PPV June sample: 28.5->100% PPV Dec sample: 35.2-63.5
Algal test (*Chlorella pyrenoidosa)*	Growth by cell density	MYT June and Dec samples: Leachate concentration lower than 12% had cell density higher than control after 8 day of experiment PPV June and Dec samples: Leachate concentration lower than 12% had cell density higher than control after 2 day of experiment
Crustacean test (*Moina macrocopa*)	Mortality	48 h LC50 MYT June sample: 15.5% MYT Dec sample: 16.3% PPV June sample: 16.4% PPV Dec sample: 19.4
Fish test (*Brachydanio rerio)*	Mortality	24-96 h LC50 MYT June sample: 2-4% MYT Dec sample: 2-4% PPV June sample: 2-4% PPV Dec sample: 2-4%

100% mortality occurred at lower leachate concentration. However, the difference between June and December samples could not be distinguished clearly. Due to the large variation of EC50 of Microtox test and the growth stimulation found in algal bioassay, the toxicity of MYT and PPV leachates could not been distinguished by these two tests.

4. NITRIFICATION OF LANDFILL LEACHATE

4.1 INTRODUCTION

Leachates from Ma Yau Tong Central (MYT) and Pillar Point Valley (PPV) Landfills had the characteristics of methanogenic leachate (Chapter 2). They were high in ammoniacal-N, low in biodegradable C and BOD:COD ratio, and had a neutral pH. Ammoniacal-N is the major pollutants of the MYT and PPV leachates with regard to its concentration and effects on environment. Ammonia levels of leachate from the MYT and PPV Landfills were within the reported toxic level which is 0.1 to 10 mg/L unionized ammonia, i.e. 14.3 to 1430 mg/L NH_x-N at 25°C and pH 7 (Environmental Protection Agency, 1985). Results of ecotoxicological study (Chapter 3) also revealed their toxic effects to water flea and zebrafish. Uncontrolled discharge may provide nutrients for algal growth and lead to eutrophication of the receiving water. Nitrification of the ammonium ions uses up dissolved oxygen and causes depletion of oxygen in the receiving water.

Physicochemical methods are usually preferred to biological method for treating 'old' leachate (Chian and DeWalle, 1976) because old leachate had small amount of the organic carbon. Lime stripping is most commonly used to remove ammoniacal-N. However, its shortcomings include the high cost of lime, formation of large quantity of alkaline sludge, requirement of high lime dosage and neutralization of effluent. Ion exchange and chlorination are also used to remove ammonia in wastewater (Eckenfelder *et al.*, 1985). In ion exchange process, natural zeolite (clinoptilolite) selectively adsorbs ammonia and releases calcium, magnesium and sodium ions, which is then regenerated by sodium or calcium hydroxide. Air stripping is required to recover the sodium and calcium hydroxide. Chlorination can

destroy ammonia completely by oxidizing ammonia to nitrogen but it also produces toxic mono- and di-chloroamines. Chlorine must be applied at very high concentration (about 8 to 10 times as high as ammonia concentration) in order to minimize the production of chloroamines.

Biological processes should be considered for treating old leachate. Nitrification and denitrification convert toxic ammonia to harmless nitrogen gas, providing an ultimate way to remove the pollutant. The cost was 30 - 55% lower than that of physicochemical methods (Eckenfelder *et al.*, 1985).

Nitrification is the key process in biological nitrogen removal. It is an aerobic biological process by autotrophic bacteria, predominantly *Nitrosomonas* and *Nitrobacter.* Nitrifiers derive energy from the oxidation of ammonia and nitrite (Eqs. 4.1 and 4.3), while using inorganic carbon for cell synthesis (Eqs. 4.2 and 4.4).

$$2NH_4^+ + 3O_2 \xrightarrow{\text{ammonia oxidizers}} 2NO_2^- + 4H^+ + 2H_2O + 116 - 168 \text{ kcal} \quad \text{Eq. 4.1}$$

$$15CO_2 + 13NH^+ \xrightarrow[\text{ammonia oxidizers}]{} 10NO_2^- + 3C_2H_7NO_2 + 4H_2O \quad \text{Eq. 4.2}$$

$$2NO_2^- + O_2 \xrightarrow{\text{nitrite oxidizers}} 2NO_3^- + 30.8 - 41.8 \text{ kcal} \quad \text{Eq. 4.3}$$

$$5CO_2 + NH_4^+ + 10NO_2^- + 2H_2O \xrightarrow[\text{nitrite oxidizers}]{} 10NO_3^- + C_5H_7NO_2 + H^+ \quad \text{Eq. 4.4}$$

However, the growth of nitrifiers is slow and susceptible to many factors such as pH, temperature, nutrients, dissolved oxygen and presence of toxic compounds (Fang *et al.*, 1993; Focht and Verstrate, 1977). To enhance nitrification, operational factors such as hydraulic retention time and nutrients should be modified to obtain an optimal rate.

It is well known that landfill leachate is low in phosphorus. However, there is little work done on the phosphate requirement of biological nitrogenous and carbonaceous treatment. A few studies investigated on the phosphate requirement for

carbonaceous removal by aerobic treatment (Mavinic, 1986). In most cases, BOD (or COD):N:P ratio of 100:5:1 is preferred for treatment system aimed at carbonaceous removal. For nitrogenous removal, it is generally agreed that low organic carbon level favors nitrification (Sharma and Ahlert, 1977). However, there is no general guideline of N: P ratio for nitrification. A wide range of phosphate concentrations from 5 to 310 mg/L PO_4^{3-}-P, were reported as optimum for the growth of nitrifying bacteria (Sharma and Ahlert, 1977; Takahashi *et al.*, 1992). It is not economic to adjust phosphate concentration to as high as 300 mg/L PO_4^{3-}-P for treatment purpose. Therefore, the treatment efficiency of nitrification at a lower phosphate level should be studied.

As nitrifiers are slow growing, longer hydraulic retention time (HRT) is required to maintain high treatment efficiency. Effective HRT depends on number of system parameters such as the concentration of nitrifying bacteria, sludge age and temperature.

Slow growing nitrifiers become less competitive than heterotrophic bacteria when organic carbon is present at high concentration (Environmental Protection Agency, 1993). This affects the efficiency of nitrification. With other parameters (temperature, pH, dissolved oxygen, etc.) held constant, the degree of nitrification decreases significantly with increasing organic loading (Carley and Mavinic, 1991; Sharma and Ahlert, 1977; Silverstein and Schroeder, 1983). It is also proposed that nitrifiers are not obligately autotrophic, and in the presence of sufficient substrate, nitrifiers will use organic carbon compounds rather than reduced nitrogen as electron donor (Robertson and Kuenen, 1992; Silverstein and Schroeder, 1983). Others proposed that inhibition was due to competition of dissolved oxygen (Hart *et al.*,

1986). Moreover, increase of organic loading result in higher rate of sludge wasting that the slow growing nitrifiers are not recycled back to the reactor and finally replaced by the fast growing hetertrophic bacteria. For leachate from old landfills, such as the MYT Landfill, the organic level is usually low and there will be no problem in organic shocking. For leachate from operating landfills, such as the PPV Landfill, there is a chance that BOD level elevates to such a high level that the rate of nitrification is affected. On the other hand, lack of heterotrophic bacteria in treatment system will lead to poor settlability of effluent. The optimum level of organic carbon should be in the range that nitrifying bacteria are not inhibited and effluent settlability are not affected.

This experiment evaluated the effectiveness of biological nitrification for eliminating ammonia in landfill leachate. The efficiency of nitrification in removing ammonia from leachate under different operation conditions was studied. The effects of different phosphate levels, hydraulic retention time and organic carbon levels on the rate of nitrification were examined. These would provide information on the design of treatment system for landfill leachate by nitrification and denitrification system.

4.2 Materials and Methods

4.2.1 Collection and analysis of leachate

Landfill leachate was collected from the Ma Yau Tong Central (MYT) Landfill in April and May 1995. The collected leachate was filled into 20-liter plastic carboys and were kept at 4°C before experiment. pH, dissolved oxygen (DO), electrical conductivity (EC), salinity, total solids (TS), alkalinity, chemical oxygen demand (COD), biochemical oxygen demand (BOD), total Kjeldahl nitrogen (TKN),

ammoniacal-N (NH_x-N), nitrite-N (NO_2^--N), nitrate-N (NO_3^--N), total Kjeldahl phosphorus (TKP) and orthophosphate-P (PO_4^{3-}-P) levels in the leachates were determined. Nitrite-N concentration of the leachates was determined by sulfanilamide method (American Public Health Association, 1992). Procedures and methods for determination of other parameters were identical to that described in Section 2.2.3 and 3.2.2.

4.2.2 Set-up of nitrification system

Bench-scale continuous flow system was set up in a laboratory for the nitrification study (Fig. 4.1). The system consisted of a 25 cm × 15 cm × 17 cm plastic tank with volume of about 6 liters as the reactor and another plastic tank of identical dimension as the clarifier. The systems were started by slowly feeding in landfill leachate, which was then seeded with returned sludge from the Shatin Sewage Treatment Plant. The systems were run for a month before the commencement of the experiment. Hydraulic retention time (HRT) of the systems was 8 days when setting-up but decreased at a rate of 2 days per week to 2 days at the start of experiment. Microbes in the added sludge were allowed to acclimate to the leachate. Dissolved oxygen (DO) of the systems was maintained at 6.5±0.5 mg/L by aerating at 2.6±0.1 L air/min. DO level higher than 2 mg/L is sufficient to maintain steady rate of nitrification (Focht and Verstrate, 1977). pH of the systems was maintained at 7 or above by adding NaOH every day. Temperature of the laboratory was controlled at 25±2°C throughout the experiment. Treated mixed liquor flowed to a clarifier, which was then allowed to settle. All of the settled sludge was recycled back to the aeration tank every day to ensure enough biomass for the reaction. There were three replicates

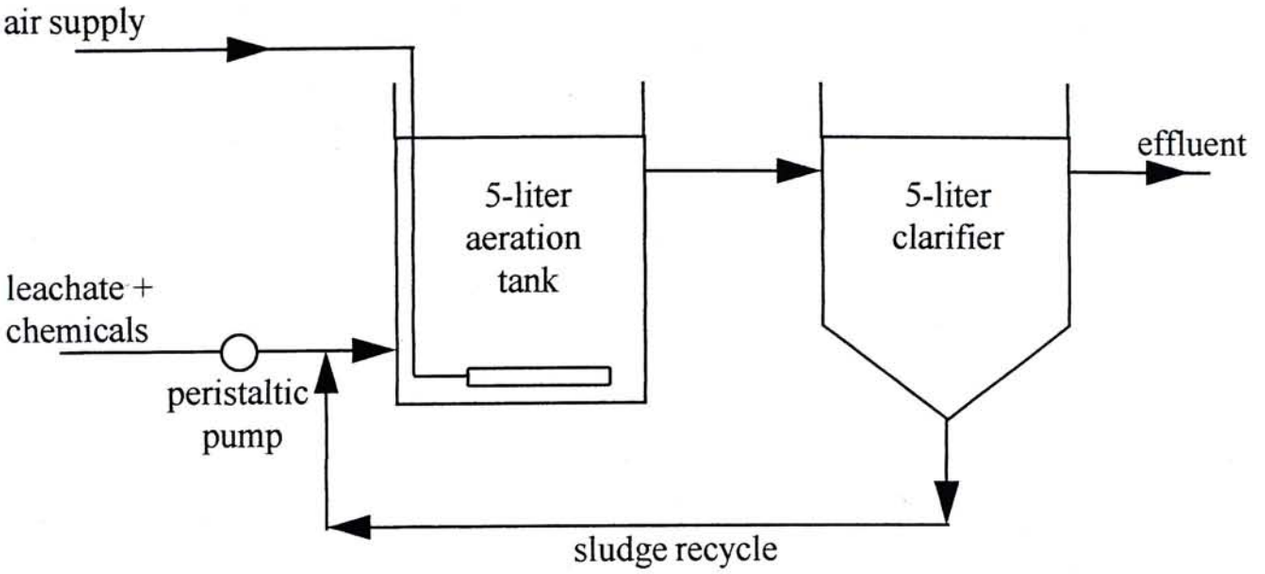


Fig. 4.1 The experimental setup for nitrification study.

for each treatment.

4.2.3 Experiment 1: Effect of additional phosphate on the rate of nitrification

Phosphate content of leachate entering the aeration tank was adjusted to 5 and 10 mg/L PO_4^{3-}-P by adding equal portion of K_2HPO_4 and Na_2HPO_4. Control was fed with unspiked leachate with a PO_4^{3-}-P concentration of 2.36 mg/L. HRT of the systems was maintained at 2 days. Control and experimental systems were fed with unspiked and phosphate-spiked leachate respectively for a week before sampling commenced. Influent into the aeration tank, mixed liquor in the aeration tank and effluent from the clarifier were sampled every two days for a period of 12 days. Ammoniacal-N, nitrite-N and nitrate-N concentrations of the settled samples were measured by phenate method, sulfanilamide method and brucine method respectively. Mixed liquor suspended solid (MLSS) and mixed liquor volatile suspended solid (MLVSS) of the aeration tank were also determined. Population of nitrifiers in the mixed liquor was determined on the 6th and 12th day of the experiment by Most Probable Number (MPN) method (Schmidt and Balser, 1982) (Appendices 3 and 4).

4.2.4 Experiment 2: Effect of HRT on the rate of nitrification

HRT of the system was set at 1, 2 and 4 days. Raw leachate (i.e. without additional phosphate) was used, as suggested by the results of Section 4.3.2. Systems were maintained at the specified flow rate for a week before sampling. Chemical and biological analyses of influent, mixed liquor and effluent were done every two days for 12 days following the methods as described in Section 4.3.3.

4.2.5 Experiment 3: Effect of additional organic carbon on the rate of nitrification

BOD of the influent was adjusted to 100 and 1000 mg/L by adding methanol (0.08 mL and 1.2 mL 98% methanol per liter of leachate respectively). No methanol was added to unspiked leachate, which contained a BOD of 42.5 mg/L. HRT was maintained at 2 days according to the results of Section 4.2.1.4. Control and experimental systems were fed with unspiked and methanol-spiked leachate respectively for a week before sampling commenced. Sampling was carried out for 12 days (Period 1). After 12 days, all systems were then fed with unspiked leachate and monitored for another 8 days (Period 2). The control tank received unspiked leachate for Periods 1 and 2. Concentrations of ammoniacal-N, nitrite-N, nitrate-N, MLSS and MLVSS were measured every two days. Populations of nitrifiers and heterotrophic bacteria were enumerated on the 6th and 12th day of Period 1. MPN method (Schmidt and Balser, 1982) was employed to enumerate nitrifiers. Spread plate method using plate count agar (American Public Health Association, 1992) was used for heterotrophic bacteria (Appendix 1).

4.2.6 Statistical analysis

The data were analyzed by nested Analysis of Variance (ANOVA) with day as nested factor at $P < 0.05$. Least Significant Difference (LSD) was calculated by Tukey's Honestly Significant Difference Test ($P < 0.05$) when significant difference was detected by ANOVA. Tests were done to evaluate the difference between influent, mixed liquor and effluent quality; and that between mixed liquor and effluent and populations of ammonia oxidizers, nitrite oxidizers and heterotrophic bacteria due to phosphate concentrations, HRT and organic carbon levels. All

statistical analyses were performed by means of SPSS (Statistical Package for Social Science) for Windows Release 6.0 of SPSS Inc.

4.3 Results and Discussion

4.3.1 Chemical properties of landfill leachate

All three batches of leachate had neutral pH, high value of EC and total solid content (Table 4.1) which indicate the high concentration of ions and particles in the leachate. The high alkalinity of landfill leachate is an advantage when nitrification is employed for leachate treatment; the high buffering capacity of raw leachate reduces the amount of alkaline required to neutralize the hydrogen ion found in nitrification.

Even though leachate had very high COD, most of portion was not biodegradable, as revealed from the low BOD:COD ratio (Table 4.1). Very high concentration of ammoniacal-N was found in all three batches of leachate which represented 74 - 84% of nitrogen in leachate. In contrast to ammoniacal-N, nitrite-N was nearly absent and nitrate-N was present at very low concentration (< 0.20 mg/L). This is due to anaerobic conditions in the landfill which present the oxidation of nitrogen compounds in the leachate.

Low concentration of phosphorus was found in leachate and less than 40% existed as phosphate form.

4.3.2 Experiment 1: Effect of additional phosphate on the rate of nitrification.

Concentration of ammoniacal-N was reduced significantly ($P < 0.05$) regardless of the influent phosphate concentration; systems with different phosphate levels had about 60% ammonia removal (Table 4.2). Nitrite concentration increased

Table 4.1 Characteristics of leachate collected from the Ma Yau Tong Central Landfill for the nitrification studies. Values shown are means and standard deviations of four replicates.

Parameters	Batch 1[a]	Batch 2[b]	Batch 3[c]
pH	7.66±0.01	7.50±0.01	7.53±0.01
DO (mg/L)	3.60±0.28	2.90±0.38	3.81±0.10
EC (μS/cm)	9290±67	9360±178	9520± 89
Salinity ($^{o}/_{oo}$)	7.13±0.48	6.75±0.65	6.00±0.00
Total solids (mg/L)	2190±30	2230±41	1670±107
Alkalinity (mg/L)	7098±99	6230±142	7220±49
COD (mg/L)	640±40	599±8	593±29
BOD (mg/L)	64.9±1.1	35.2±1.7	42.5±5.1
BOD/COD	0.10	0.04	0.04
TKN (mg/L)	1010±31	1020±38	1041±51
NH_x-N (mg/L)	642±12	774±17	871±62
NO_2^--N (mg/L)	< 0.001	< 0.001	< 0.001
NO_3^--N (mg/L)	0.16±0.02	0.10± 0.01	0.19± 0.10
TKP (mg/L)	6.59±1.48	5.43±0.80	4.66±0.26
PO_4^{3-}-P (mg/L)	2.36±0.02	1.20±0.30	1.74±0.44

[a] Leachate collected in April 1995 for Experiment 1: Effect of additional phosphate on the rate of nitrification.

[b] Leachate collected in May 1995 for Experiment 2: Effect of HRT on the rate of nitrification.

[c] Leachate collected in May 1995 for Experiment 3: Effect of additional organic carbon on the rate of nitrification.

Table 4.2 Quality of influent, mixed liquor and effluent from systems fed with (1) unspiked leachate containing 2.36 mg/L PO_4^{3-}-P (control), (2) leachate spiked with phosphate to 5 mg/L P and (3) leachate spiked with phosphate to 10 mg/L P. Values shown are means and standard deviations of three replicates measured every two days for 12 days.

	Concentration of PO_4^{3-}-P			LSD[a]
	Control	5 mg/L	10 mg/L	
NH_x-N (mg/L)				
influent	631±37	654±36	640±41	-
mixed liquor	241±36	235±38	251±35	NS
effluent	247±42	226±52	255±44	NS
LSD[b]	31	34	32	
NO_2^--N (mg/L)				
influent ($\times 10^{-3}$)	0.12±0.03	0.10±0.05	0.10±0.06	-
mixed liquor	0.43±0.04	0.52±0.12	0.54±0.08	0.07
effluent	0.45±0.04	0.51±0.13	0.50±0.11	NS
LSD[b]	0.05	0.02	0.04	
NO_3^--N (mg/L)				
influent	0.16±0.12	0.15±0.10	0.18±0.09	-
mixed liquor	115±60	97±59	105±47	NS
effluent	108±42	132±75	132±78	NS
LSD[b]	34	68	43	
MLSS (mg/L)	337±110	264±68	380±159	95
MLVSS (mg/L)	294±93	225±59	324±139	82

[a] LSD denotes least significant difference ($P < 0.05$) in quality due to difference in phosphate levels by the Tukey's Honestly Significant Difference Test.

[b] LSD denotes least significant difference ($P < 0.05$) between quality of influent, mixed liquor and effluent by the Tukey's Honestly Significant Difference Test.

NS denotes no significant difference ($P > 0.05$) according to ANOVA.

significantly ($P < 0.05$) from < 0.001 mg/L to about 0.5 mg/L in the mixed liquor and effluent. Nitrate concentration was elevated 650 - 700 folds, from smaller than 0.2 mg/L to 97 - 115 mg/L in mixed liquor and 108 - 132 mg/L in effluent. For these three forms of inorganic nitrogen, there were no significant differences ($P > 0.05$) between concentrations in the mixed liquor and effluent (Figs. 4.2 - 4.4) except nitrite concentration in mixed liquor. This indicates that oxidation of ammonium and nitrate occurred in the aeration tank but no in the clarifier. The low nitrite concentrations in the mixed liquor and effluent suggests that nitrite oxidizers was not inhibited (Anthonisen *et al.*, 1976) at low phosphate level.

Although all of the settled solids were recycled back to aeration tank every day, MLSS and MLVSS could only be maintained at 264 - 380 and 225 - 324 mg/L respectively in all systems (Table 4.3), which were lower than that found in activated sludge system. In local sewage treatment plant, MLSS of activated sludge process is maintained at 2000 - 3500 mg/L (Wu, 1994) which is much higher than that in the present study. Little heterotrophic bacteria were present in this nitrification system to form matrix for floc formation. The low solid (microorganism) concentration may limit the rate of nitrification. To increase the ammonia removal efficiency, increase of retention time or solid concentration may be required, e.g. by the addition of sludge from an external source.

No significant difference in nitrifier populations was found between different phosphate levels (Table 4.4). Population of ammonia oxidizers was 10^5 folds as high as that of nitrite oxidizers. Lower number of nitrite oxidizers were also found in other nitrification systems (Sharma and Ahlert, 1977). Oxidation of ammonia can release more energy than nitrite oxidation (Eqs 4.1 and 4.3). A greater biomass of ammonia oxidizers is produced per mole of nitrogen oxidized if energy utilization efficiency of

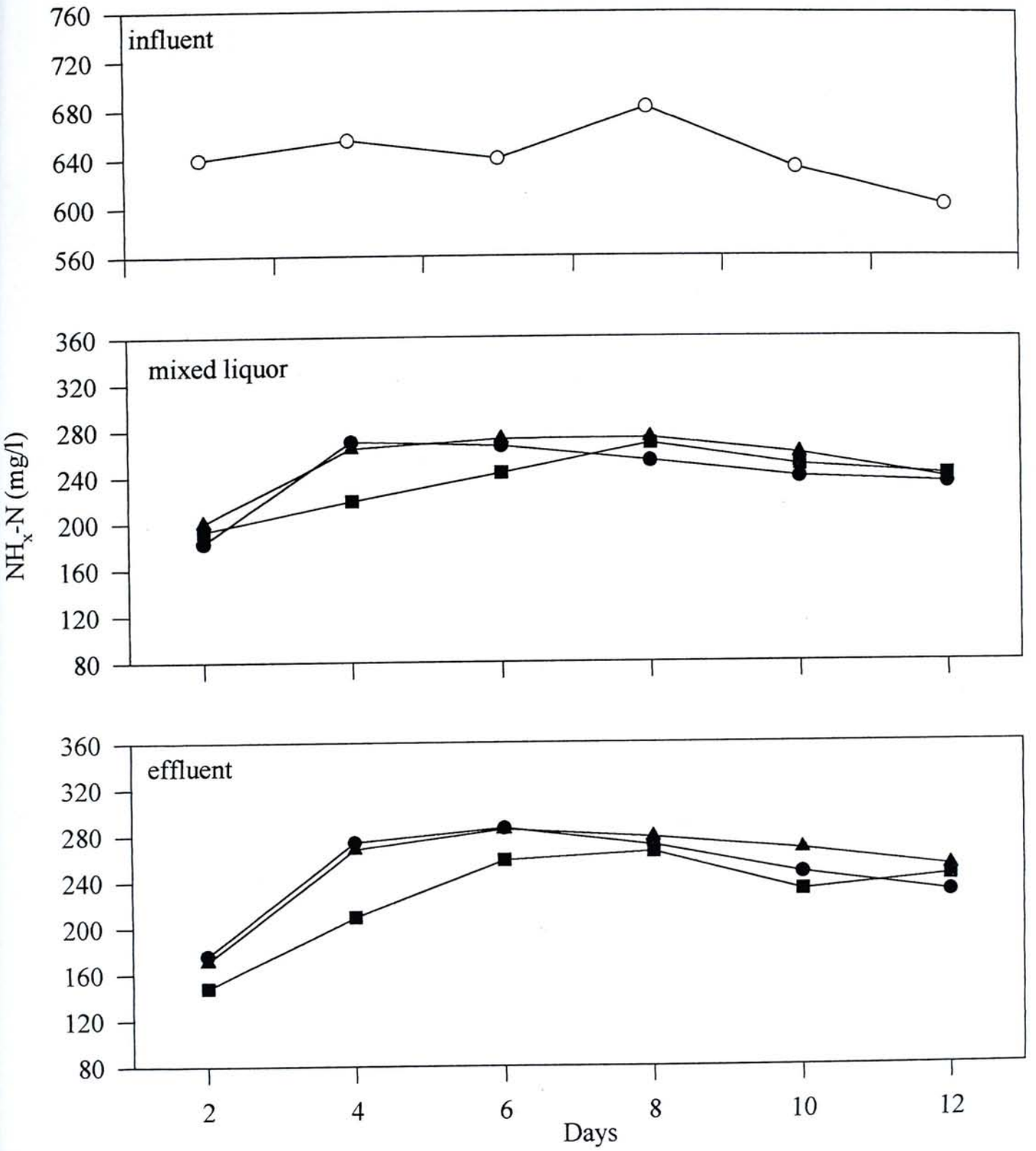


Fig. 4.2 Ammoniacal-N concentrations of influent, mixed liquor and effluent from systems fed with (1) unspiked leachate containing 2.36 mg/L P(control) (●), (2) leachate spiked with phosphate to 5 mg/L P (■) and (3) leachate spiked with phosphate to 10 mg/L P (▲). Values are means of three replicates measured every two days for 12 days. No significant difference was found between different phosphate levels by ANOVA at P = 0.05.

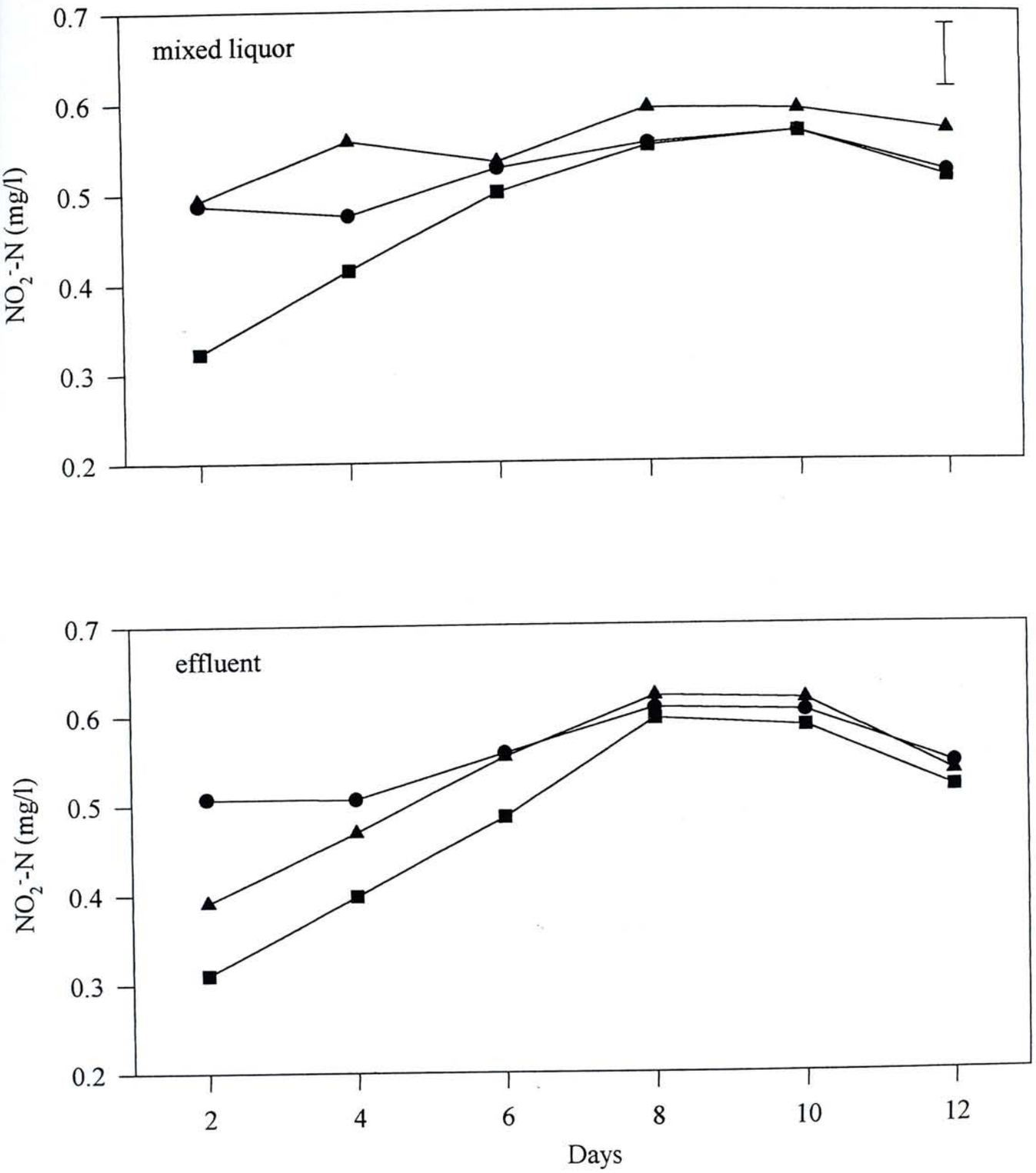


Fig. 4.3 Nitrite-N concentrations of mixed liquor and effluent from systems fed with (1) unspiked leachate containing 2.36 mg/L P (control) (●), (2) leachate spiked with phosphate to 5 mg/L P (■) and (3) leachate spiked with phosphate to 10 mg/L P (▲). Values are means of three replicates measured every two days for 12 days. Vertical bar denotes LSD of concentration in mixed liquor by Tukey's Honestly Significant Difference Test at $P = 0.05$. No significant difference in concentration in effluent was found between different phosphate levels by ANOVA at $P < 0.05$.

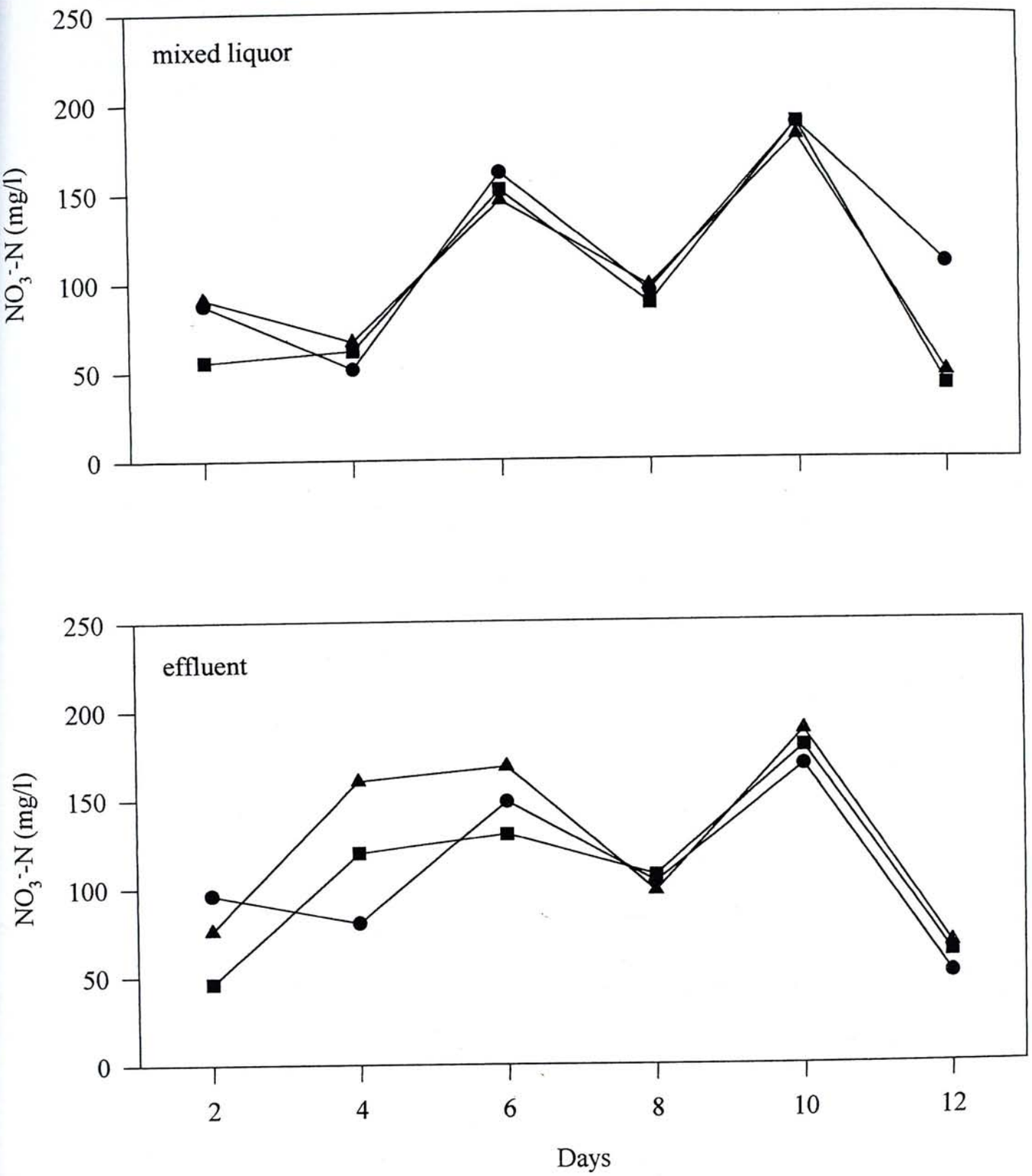


Fig. 4.4 Nitrate-N concentrations of mixed liquor and effluent from systems fed with (1) unspiked leachate containing 2.36 mg/L P (control) (●), (2) leachate spiked with phosphate to mg/L P (■) and (3) leachate spiked with phosphate to 10 mg/L P (▲). Values are means of three replicates measured every two days for 12 days. No significant difference was found between different phosphate levels by ANOVA at $P < 0.05$.

Table 4.3 Ammonia removal of systems fed with (1) unspiked leachate containing 2.36 mg/L PO_4^{3-}-P, (control) (2) leachate spiked with phosphate to 5 mg/L P and (3) leachate spiked with phosphate to 10 mg/L P. Values shown are means and standard deviations of three replicates measured every two days for 12 days.

	Influent-mixed liquor	Influent-effluent
NH_x-N removed (%)[a]		
control	61.8±5.5	60.8±6.6
5 mg/L	64.1±5.8	65.5±7.8
10 mg/L	60.8±5.7	60.1±6.9
LSD	NS	NS
NH_x-N removed/MLSS (mg NH_x-N/mg MLSS/d)[b]		
control	0.65±0.24	0.64±0.25
5 mg/L	0.83±0.18	0.85±0.19
10 mg/L	0.61±0.26	0.60±0.28
LSD	0.20	0.19
NH_x-N removed/MLVSS (mg NH_x-N/mg MLVSS/d)[c]		
control	0.73±0.26	0.72±0.27
5 mg/L	0.98±0.22	1.00±0.24
10 mg/L	0.72±0.32	0.72±0.34
LSD	0.22	0.23

[a] NH_x -N removed (%)

$$= \frac{\text{influent conc. - mixed liquor or effluent conc.}}{\text{influent conc.}} \times 100\%$$

[b] NH_x-N removed/MLSS (mg NH_x-N/mg MLSS/day)

$$= \frac{(\text{influent conc. - mixed liquor or effluent conc.}) \times \text{flow rate}}{\text{MLSS conc.} \times \text{reactor volume}}$$

[c] NH_x-N removed/MLVSS (mg NH_x-N/mg MLVSS/day)

$$= \frac{(\text{influent conc. - mixed liquor or effluent conc.}) \times \text{flow rate}}{\text{MLVSS conc.} \times \text{reactor volume}}$$

LSD denotes least significant difference ($P < 0.05$) in removal rate due to differences in phosphate levels by the Tukey's Honestly Significant Difference Test.

NS denotes no significant difference ($P > 0.05$) according to ANOVA.

Table 4.4 Populations of ammonia oxidizers and nitrite oxidizers in mixed liquor of systems fed with unspiked leachate containing 2.36 mg/L PO_4^{3-}-P(control), (2) leachate spiked with phosphate to 5 mg/L P and (3) leachate spiked with phosphate to 10 mg/L P. Values shown are means and standards deviations of three replicates measured on the 6th and 12th day of the experiment.

	Ammonia oxidizers ($\times 10^5$ MPN/mL)	Nitrite oxidizers (MPN/mL)
6th day		
control	3.30±0.00	16.5±5.0
5 mg/L	2.30±1.41	7.80±0.00
10 mg/L	1.05±0.36	8.75±6.01
12th day		
control	5.95±1.48	2.00±0.00
5 mg/L	4.10±1.13	2.00±0.00
10 mg/L	5.37±2.34	16.5±5.0

N.B. No significant differences ($P > 0.05$) between different phosphate levels at the 6th or 12th day for both types of nitrifiers according to ANOVA.

ammonia oxidizers and nitrite oxidizers is equal.

Landfill leachate is usually deficient in phosphorus. In most aerobic treatability test for leachate (carbonaceous or nitrogenous removal), addition of phosphate is a usual practice (Table 4.5). Leachates used in other studies had higher BOD and those studies mostly focused on organic carbon removal. Usually, C (COD or BOD): P ratio was maintained at 100:1 (Bull *et al.*, 1983; Robinson and Maris, 1983 and 1985; Young *et al.*, 1987). This ratio is usually employed for organic carbon removal of wastewater treatment. For study of wastewater treatment by nitrification, no general guideline of phosphate requirement was established. Phosphorus concentrations of some nitrification studies are shown in Table 4.6.

In this study, phosphate levels of 5 and 10 mg/L were chosen for comparison. Phosphate concentration of control system was 2.36 mg/L PO_4^{3-}-P. However, there was no difference in rate of nitrification with respect to ammonia removal (Table 4.3), nitrate production (Table 4.2) as well as population of nitrifiers (Table 4.4). The difference of ammonia removal of systems with and without phosphate addition was less than 5% (Table 4.3). Some factors other than phosphate concentration may have limited the rate of nitrification. Therefore, in the next two experiments, no phosphate would be added to the systems.

This is clearly an advantage as no additional phosphate is required for the nitrification of landfill leachate. This would decrease the cost of treatment and simplify the operation process. Phosphorus, along with nitrogen, is the major nutrients causing eutrophication. In the nitrification study of landfill leachate, addition of phosphorus to 25 mg/L in influent resulted in effluent with phosphorus concentration in the 10 - 20 mg/L range (Knox, 1985). Unless all the added phosphate would be removed along treatment process, it poses another pollution

Table 4.5 Phosphorus amendment employed for landfill leachate treatment.

Organic concentration	Phosphorus amendment	System	Reference
BOD = 4000 mg/L	$(NH_4)_3PO_4$ to BOD:P = 100:1	suspended growth, batch study	Bull *et al.*, 1983
COD = 5000 mg/L	Na_2HPO_4 & NaH_2PO_4 to COD:P < 100:1	suspended growth, bench scale	Robinson and Maris, 1983
BOD = 8143-12468 mg/L	H_3PO_4, conc. not stated, also employed as neutralizing agent after liming	suspended growth, pilot scale	Keenan *et al.*, 1984
COD = 11900 mg/L	Na_2HPO_4 & NaH_2PO_4 to COD:P < 100:1	suspended growth, bench scale	Robinson and Maris, 1985
BOD = 30500 mg/L	BOD:P=1000:1	suspended growth, batch scale	Gaudy *et al.*, 1986
BOD = 1663-10721 mg/L	H_3PO_4 to BOD:P=100:1	aeration lagoon, pilot scale	Young *et al.*, 1987
BOD = 87000-240000 mg/L	H_3PO_4, conc. not stated	aeration lagoon, field scale	Robinson, 1992
BOD = 135-384 mg/L	COD:P=1000:1	suspended growth, bench scale	Carville and Robinson, 1991
BOD = 26 mg/L	NaH_2PO_4 to 10 mg/L P	rotating biological contactor, bench scale	Spengel and Dzombak, 1991

Table 4.6 Phosphate concentration of influent of nitrification treatment processes.

System	Wastewater	Phosphorus concentration	Reference
Sequencing biological reactor	artifical wastewater	12 mg/L PO_4^{3-}-P	Alleman and Irvine, 1980
		4.89-9.79 mg/L PO_4^{3-}-P	Silverstein and Schroeder, 1983
		3.9-6.2 mg/L PO_4^{3-}-P	Jone *et al.*, 1990
	settled sewage	4.69 mg/L PO_4^{3-}-P	Tam *et al.*, 1992
Activated sludge system	chemical plant wastewater	> 5 mg/L total P	Ford *et al.*, 1980
	landfill leachate	TOC:P = 100:1	Knox, 1985
	oil refinery wastewater	5 mg/L PO_4^{3-}-P	Fang *et al.*, 1993
Trickling filter	landfill leachate	TOC:P = 100:1	Knox, 1985
Upflow and downflow submerged filter	sewage	8.7 mg/L total P	Jansen *et al.*, 1994

problem to the environment.

4.3.3 Experiment 2: Effect of HRT on the rate of nitrification

Different flow rates and hence different volume of leachate will have different amount of ammonia going into the systems. When the rate of nitrification is compared, both the effluent quality (Table 4.7) and the rate of ammonia removal, i.e. total amount removed per day and the amount removed per unit weight of solids (MLSS and MLVSS) per day must be employed as indices for assessing system efficiency (Table 4.8). When comparing the concentration of ammonia in the mixed liquor and effluent, 1-day system had higher concentration than the 2- and 4-day systems, which did not differ significantly (Table 4.7 and Fig. 4.5). However, in terms of total ammonia removed and ammonia removed per MLSS or per MLVSS per day, system with HRT of 1 day was higher than that of 2 days which was in turn higher than that of 4 days (Table 4.8). Ammonia oxidizers have a higher rate of oxidation of ammonia when input rate of ammonia increases. However, shorter retention time and thus higher flow rate may result in the incomplete treatment of leachate. It can be seen that in the one-day retention system, although high oxidation rate was obtained (1520 mg NH_x-N/d), effluent with poor quality (higher NH_x-N content) was produced. As the increase of HRT from 2 to 4 days did not improve effluent quality in terms of ammonia concentration (Table 4.8), HRT of two days appears to be optimal. However, the factor(s) that limited the removal efficiency for system of HRT of 4 days was not known. Possible factors included high nitrate concentration (Table 4.7) and low microbial biomass (Table 4.9).

Two-day and four-day systems had similar concentration of nitrite in the mixed liquor and effluent and were significantly higher than that found on one-day

Table 4.7 Quality of influent, mixed liquor and effluent from systems with HRT of 1, 2 and 4 days. Values shown are means and standard deviations of three replicates measured every two days for 12 days.

	HRT			LSD[a]
	1 day	2 days	4 days	
NH_x-N (mg/L)				
influent	793±97	753±121	767±94	-
mixed liquor	431±84	333±40	322±44	47
effluent	413±57	343±49	349±49	41
LSD[b]	65	58	53	
NO_2^--N (mg/L)				
influent ($\times 10^{-3}$)	0.21±0.12	0.05±0.04	0.06±0.05	-
mixed liquor	0.43±0.08	0.51±0.01	0.50±0.03	0.04
effluent	0.50±0.07	0.53±0.03	0.54±0.07	0.05
LSD[b]	0.05	0.02	0.04	
NO_3^--N (mg/L)				
influent	0.12±0.09	0.10±0.04	0.09±0.05	-
mixed liquor	105±22	134±23	164±25	20
effluent	120±25	154±26	174±55	30
LSD[b]	16	16	28	
MLSS (mg/L)	134±62	145±22	241±127	58
MLVSS (mg/L)	123±57	132±19	215±117	53

[a] LSD denotes least significant difference ($P < 0.05$) in quality due to difference in HRT by the Tukey's Honestly Significant Difference Test.

[b] LSD denotes least significant difference ($P < 0.05$) between quality of influent, mixed liquor and effluent by the Tukey's Honestly Significant Difference Test.

Table 4.8 Ammonia removal of systems with HRT of 1, 2 and 4 days. Values shown are means and standard deviations of three replicates measured every two days for 12 days.

	Influent-mixed liquor	Influent-effluent
NH_x-N removed (%)[a]		
HRT = 1 day	45.5±9.4	47.6±6.7
HRT = 2 days	55.3±4.9	54.0±4.7
HRT = 4 days	57.9±4.3	54.3±5.7
LSD	5.3	4.7
Total NH_x-N removed (mg/d)[b]		
HRT = 1 day	1450±376	1520±343
HRT = 2 days	846±193	818±179
HRT = 4 days	445±73	418±78
LSD	197	181
NH_x-N removed/MLSS (mg NH_x-N/mg MLSS/d)[c]		
HRT = 1 day	2.69±1.09	2.80±1.14
HRT = 2 days	1.50±0.38	1.46±0.37
HRT = 4 days	0.54±0.30	0.52±0.31
LSD	0.53	0.55
NH_x-N removed/MLVSS (mg NH_x-N/mg MLVSS/d)[d]		
HRT = 1 day	2.87±1.15	3.00±1.25
HRT = 2 days	1.65±0.42	1.619±0.40
HRT = 4 days	0.59±0.30	0.56±0.31
LSD	0.55	0.60

[a] NH_x -N removed (%)

$$= \frac{\text{influent conc. - mixed liquor or effluent conc.}}{\text{influent conc.}} \times 100\%$$

[b] Total NH_x-N removed (mg/d)

= (influent conc.- mixed liquor or effluent conc.) × flow rate

[c] NH_x-N removed/MLSS (mg NH_x-N/mg MLSS/day)

$$= \frac{\text{(influent conc. - mixed liquor or effluent conc.) × flow rate}}{\text{MLSS conc. × reactor volume}}$$

[d] NH_x-N removed/MLVSS (mg NH_x-N/mg MLVSS/day)

$$= \frac{\text{(influent conc. - mixed liquor or effluent conc.) × flow rate}}{\text{MLVSS conc. × reactor volume}}$$

LSD denotes least significant difference ($P < 0.05$) in removal rate due to difference in HRT by the Tukey's Honestly Significant Difference Test.

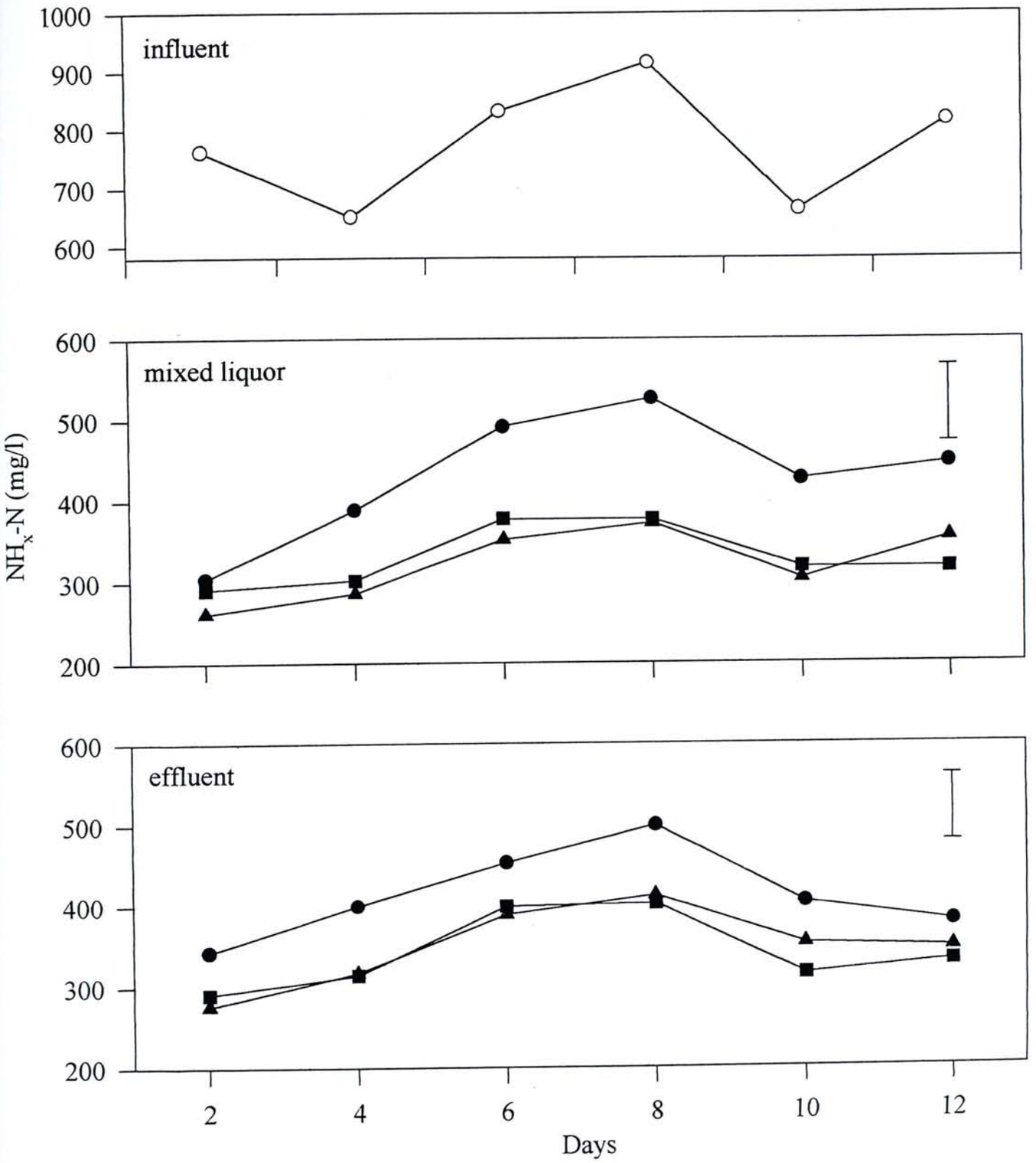


Fig. 4.5 Ammoniacal-N concentrations of influent, mixed liquor and effluent from systems with HRT of 1 (●), 2 (■) and 4 (▲) days. Values are means of three replicates measured every two days for 12 days. Vertical bars denote LSD by the Tukey's Honestly Significant Difference Test at $P = 0.05$.

Table 4.9 Population of ammonia oxidizers and nitrite oxidizers in mixed liquor of systems with HRT of 1, 2 and 4 days. Values shown are means and standard deviations of three replicates measured on 6th and 12th day of the experiment.

	Ammonia oxidizers ($\times 10^5$ MPN/mL)	Nitrite oxidizers (MPN/mL)
6th day		
HRT = 1 day	0.25±0.04	24.0±0.0
HRT = 2 days	0.26±0.16	35.3±12.7
HRT = 4 days	0.44±0.09	35.3±12.7
12th day		
HRT = 1 day	0.29±0.29	26.3±11.6
HRT = 2 days	0.30±0.23	27.0±5.2
HRT = 4 days	0.59±0.17	30.0±5.2

N.B. No significant differences ($P > 0.05$) between different HRT at the 6th or 12th day for both types of nitrifiers according to ANOVA.

system (Table 4.7 and Fig. 4.7). The longer the HRT, the more the nitrate in the mixed liquor and effluent (Table 4.7 and Fig. 4.8).

MLSS and MLVSS in the system of 4-day retention time were about twice as high as those of the other two systems (Fig. 4.9). Decreasing flow rate reduced the amount of solids lost along with the effluent. However, there may not be a corresponding increase in the populations of ammonia and nitrite oxidizers as suggested by the fact that their population in 4-day system were not statistically higher ($P > 0.05$) than those of the other two systems (Table 4.9). Similar population of nitrifiers may accounted for similar ammonia removal efficiency of systems with HRT of 2 and 4 days. Increase in solid content but not the nitrifier population in the 4-day system did not significantly improve system performance when compared with the 2-day system.

Systems of 1- and 2-day HRT had similar level of solid concentration (Table 4.7) and nitrifier population (Table 4.9). However, the total amount of ammonia removed by the 1-day system (1520 mg/d NH_x-N) was about twice as high as the 2-day one (818 mg/d NH_x-N) (Fig. 4.6). This suggests that nitrifiers had the ability to remove ammonia at a faster rate. When influent with higher ammonia concentration is fed into the 2-day system, ammonia concentration of effluent can still be maintained at similar level.

As system with HRT of 2 days had similar ammonia removal efficiency to the 4-day system and could treat higher volume of leachate. In the next experiment on the effect of additional organic carbon, HRT of 2 days would be employed.

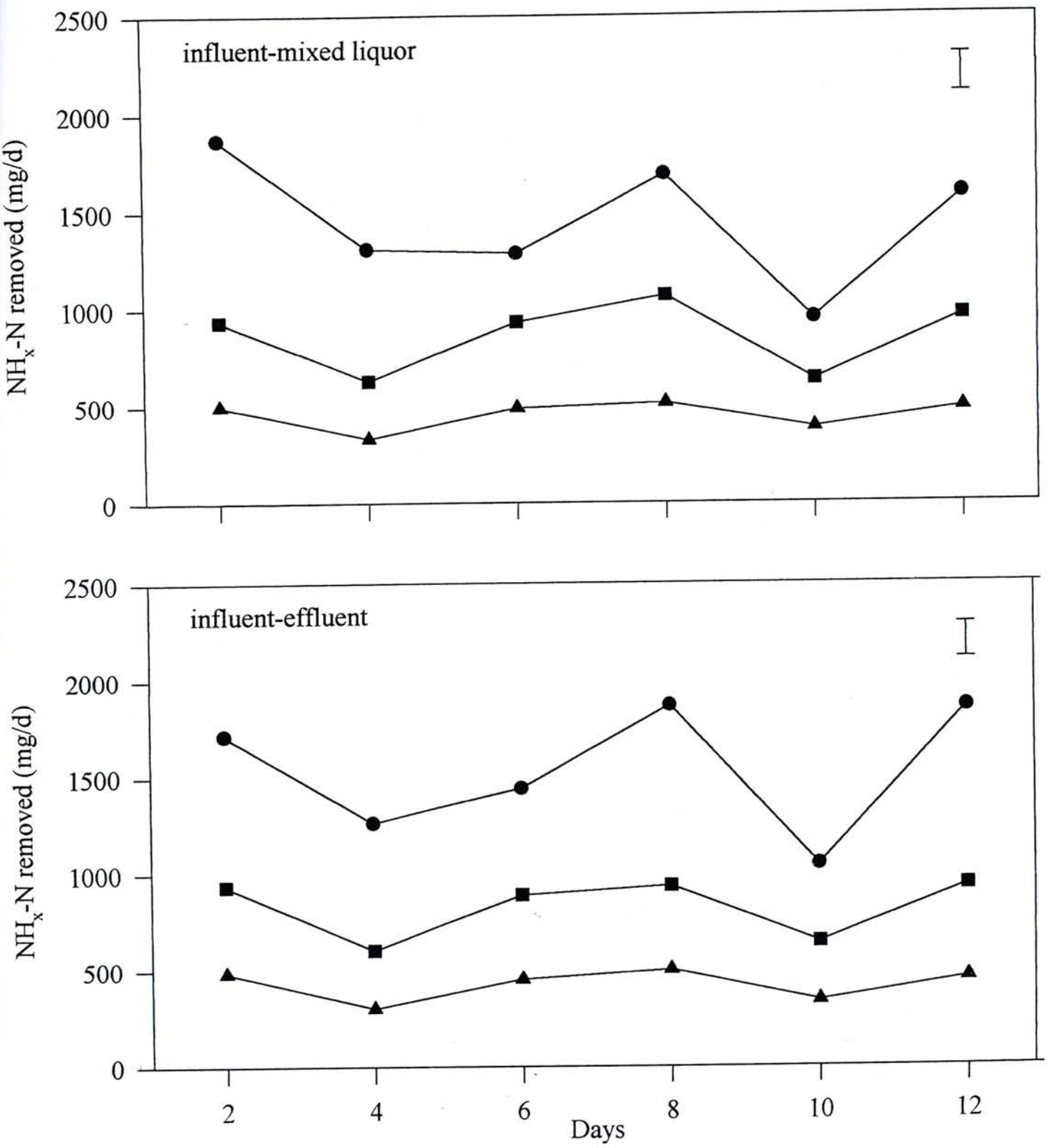


Fig. 4.6 Total ammoniacal-N removed per day by systems with HRT of 1 (●), 2 (■) and 4 (▲) days. Values are means of three replicates measured every two days for 12 days. Vertical bars denote LSD by the Tukey's Honestly Significant Difference Test at P = 0.05.

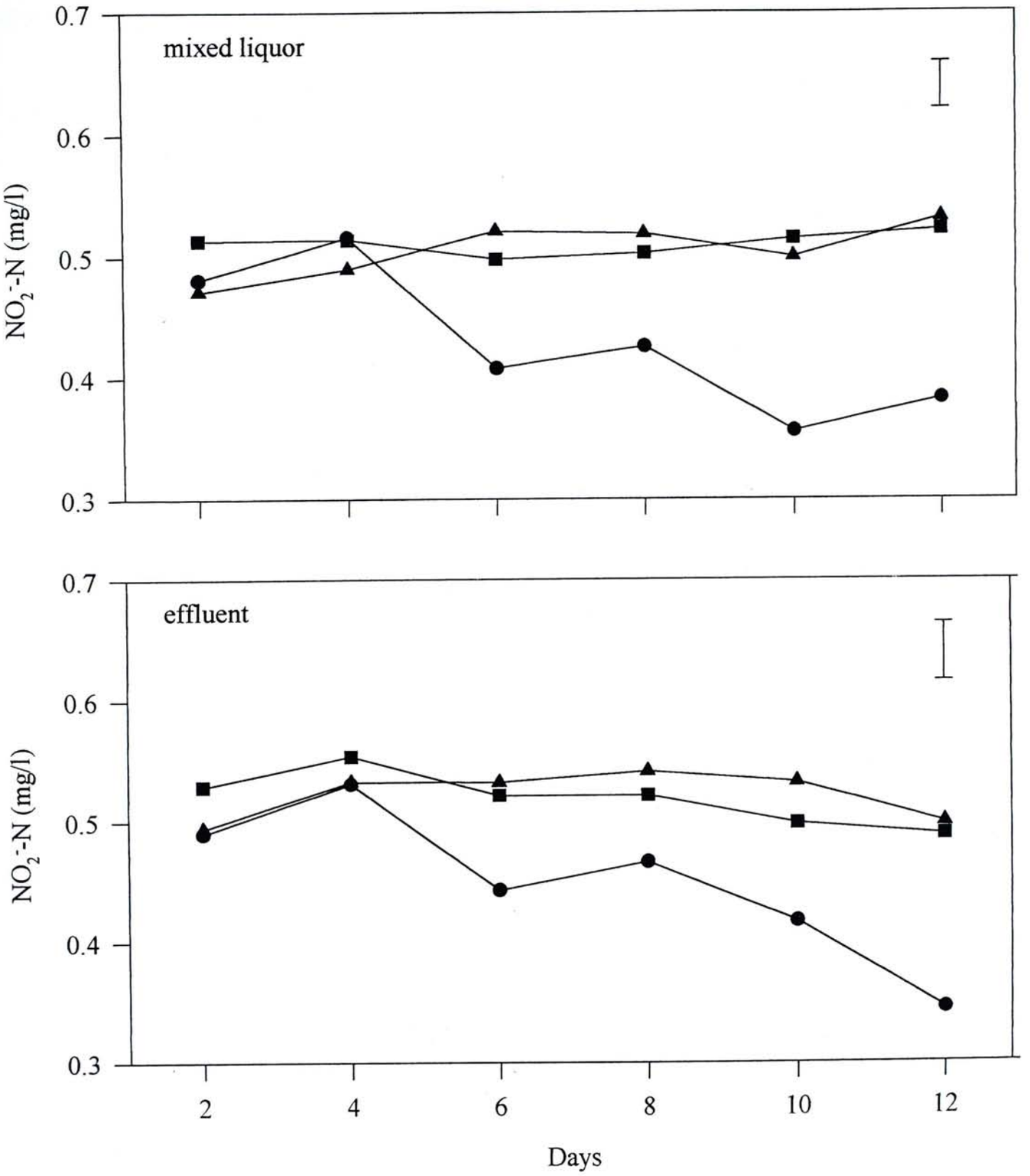


Fig. 4.7 Nitrite-N concentrations of mixed liquor and effluent from systems with HRT of 1 (●), 2 (■) and 4 (▲) days. Values are means of three replicates measured every two days for 12 days. Vertical bars denote LSD by the Tukey's Honestly Significant Difference Test at $P = 0.05$.

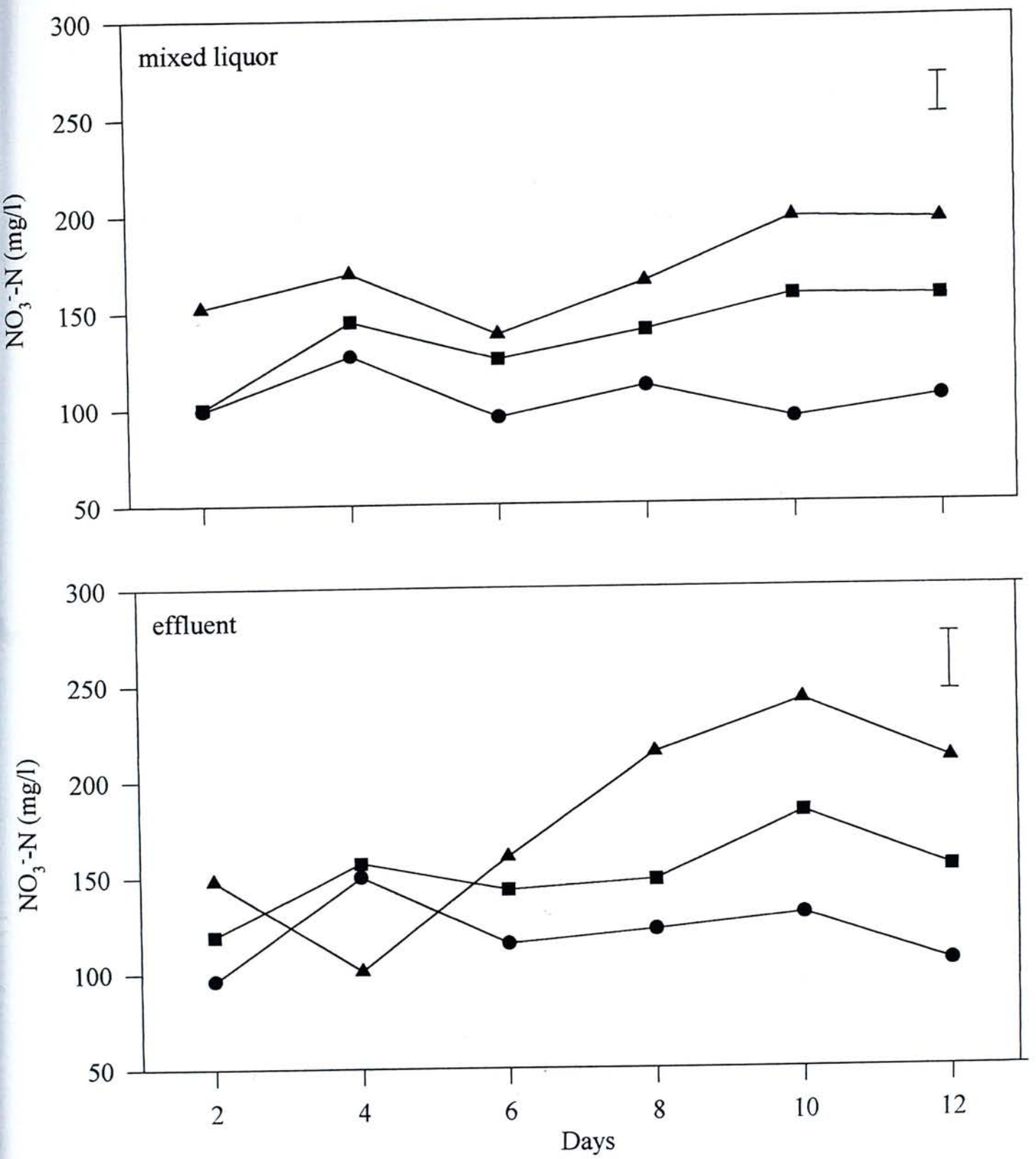


Fig. 4.8 Nitrate-N concentrations of mixed liquor and effluent from systems with HRT of 1 (●), 2 (■) and 4 (▲) days. Values are means of three replicates measured every two days for 12 days. Vertical bars denote LSD by the Tukey's Honestly Significant Difference Test at $P = 0.05$.

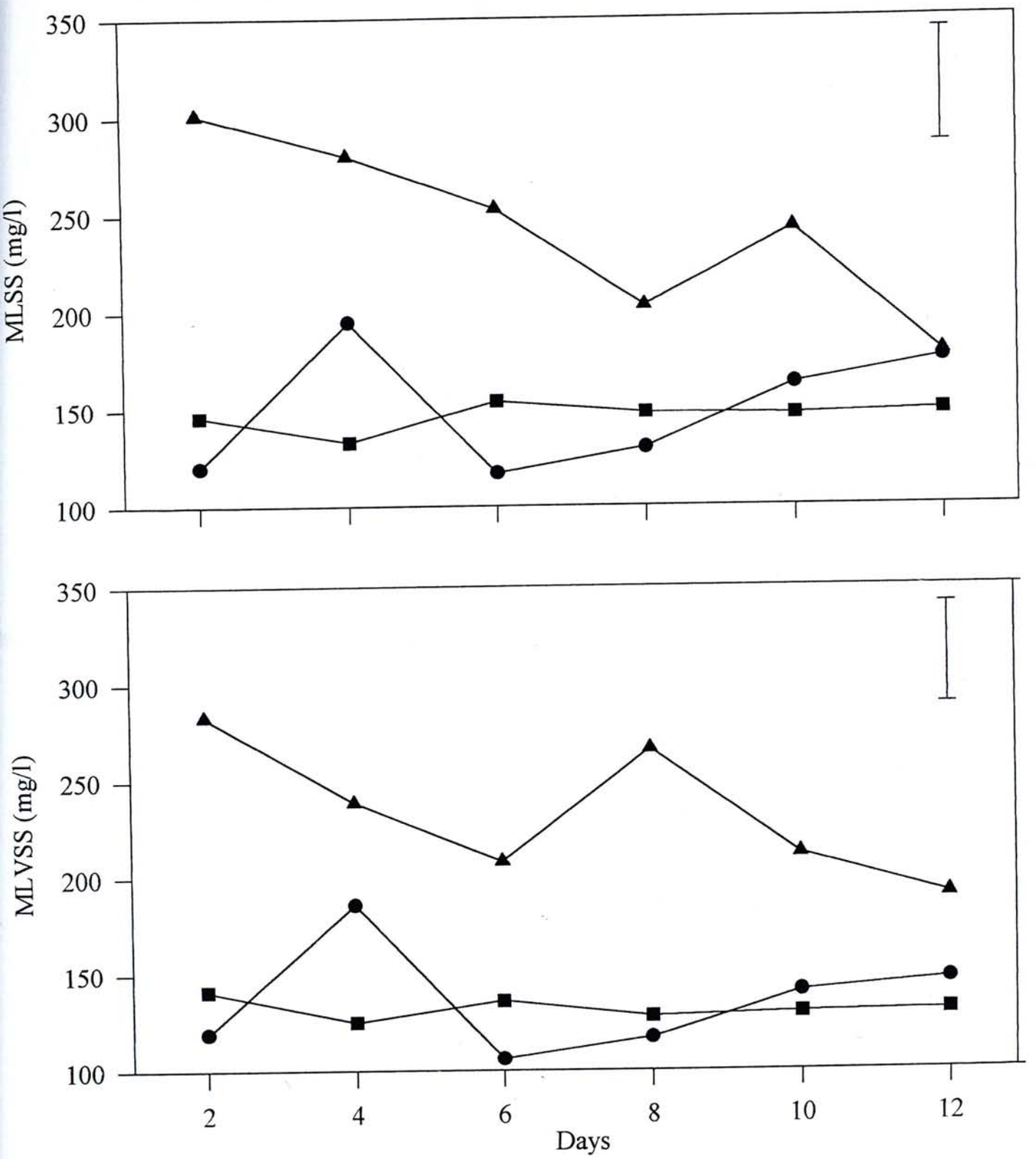


Fig. 4.9 MLSS and MLVSS of systems with HRT of 1 (●), 2 (■) and 4 (▲) days. Values are means of three replicates measured every two days for 12 days. Vertical bars denote LSD by the Tukey's Honestly Significant Difference Test at $P < 0.05$.

4.3.4 Experiment 3: Effect of additional organic carbon on the rate of nitrification

For system fed with influent containing 1000 mg/L BOD, ammonia concentration of effluent was significantly lower than that of the mixed liquor in the aeration tank during Period 1 of the experiment (Table 4.10). Loss of ammonia occurred in the clarifier of system with the highest organic carbon loading. Nitrifiers are less competitive than heterotrophic bacteria in low oxygen environment (Hart *et al.*, 1986). Systems with low organic carbon loading (42.5 and 100 mg/L) had similar concentrations of ammonia and nitrate in the mixed liquor and effluent (Table 4.10). Nitrification is hindered as a result of oxygen stress in the clarifier where there is no forced aeration. Nitrification, if occurred, was not the major cause of ammonia reduction in clarifier of system with influent containing 1000 mg/L BOD. Heterotrophic bacteria, which are less susceptible to oxygen stress, assimilate ammonia for biomass production. High organic loading can promote ammonia assimilation by the heterotrophic bacteria in denitrification-nitrification (Carley and Mavinic, 1991). In terms of ammonia removal, system with influent of 1000 mg/L BOD exhibited the highest efficiency when ammonia concentration of the mixed liquor or effluent was considered (Fig. 4.10).

Concentration of nitrate (Fig. 4.11) and nitrite (Fig. 4.12) of mixed liquor and effluent of system which received influent containing 1000 mg/L BOD were only half of the other two systems in Period 1. This is possible due to either lower rate of production by nitrification or higher rate of removal by denitrification. The half-saturation coefficient of ammonia oxidation rate by *Nitrosomonas* at 25°C is 0.6 - 3.6 mg/L NH_4^+-N (Grady and Lim, 1980). Ammoniacal-N concentration of the mixed liquor of the system with BOD of 1000 mg/L was about 300 mg/L (Table 4.10). It

Table 4.10 Quality of influent, mixed liquor and effluent from systems fed with (1) unspiked leachate containing 42.5 mg/L BOD (control), (2) leachate spiked with methanol to 100 mg/L BOD and (3) leachate spiked with methanol to 1000 mg/L BOD in Period 1 (the first 12 days). Values are means and standard deviations of three replicates measured every two days for 12 days.

	Period 1			LSD[a]
	Control	100 mg/L BOD	1000 mg/L BOD	
NH_x-N (mg/L)				
influent	860±62	894±49	894±73	-
mixed liquor	297±34	334±41	280±51	34
effluent	318±29	351±39	227±74	40
LSD[b]	53	35	36	
NO_2^--N (mg/L)				
influent ($\times 10^{-3}$)	0.18±0.06	0.22±0.06	0.20±0.08	-
mixed liquor	0.60±0.03	0.61±0.04	0.30±0.13	0.06
effluent	0.65±0.45	0.65±0.47	0.31±0.14	0.07
LSD[b]	0.09	0.03	0.03	
NO_3^--N (mg/L)				
influent	0.18±0.02	0.22±0.03	0.20±0.08	-
mixed liquor	162±26	178±28	81±32	28
effluent	170±31	187±30	83±33	24
LSD[b]	21	19	19	
MLSS (mg/L)	142±66	196±32	377±153	81
MLVSS (mg/L)	122±59	169±28	320±127	68

[a] LSD denotes least significant difference ($P < 0.05$) in quality due to difference in BOD by the Tukey's Honestly Significant Difference Test.

[b] LSD denotes least significant difference ($P < 0.05$) between quality of influent, mixed liquor and effluent by the Tukey's Honestly Significant Difference Test.

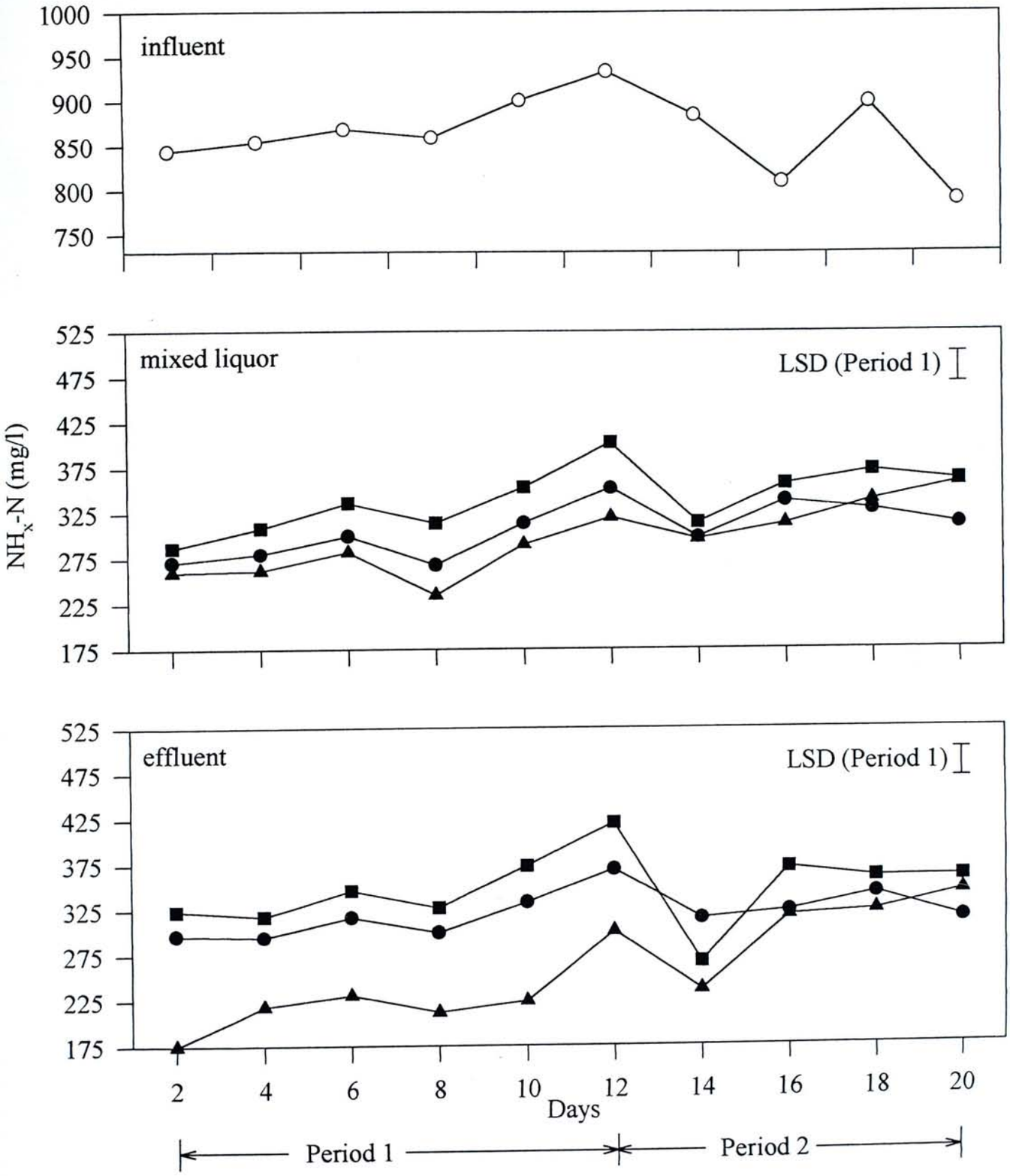


Fig. 4.10 Ammoniacal-N concentrations of influent, mixed liquor and effluent from systems fed with leachate with (1) unspiked leachate containing 42.5 mg/L BOD (control) (●), (2) leachate spiked with methanol to 100 mg/L BOD (■) and (3) leachate spiked with methanol to 1000 mg/L BOD (▲) for 12 days (Period 1) and then with unspiked leachate for 8 days (Period 2). Values are means of three replicates measured every two days. Vertical bars denote LSD between different BOD level by the Tukey's Honestly Significant Difference Test during Period 1 at $P = 0.05$. No significant difference was found between different BOD levels by ANOVA during Period 2 at $P < 0.05$.

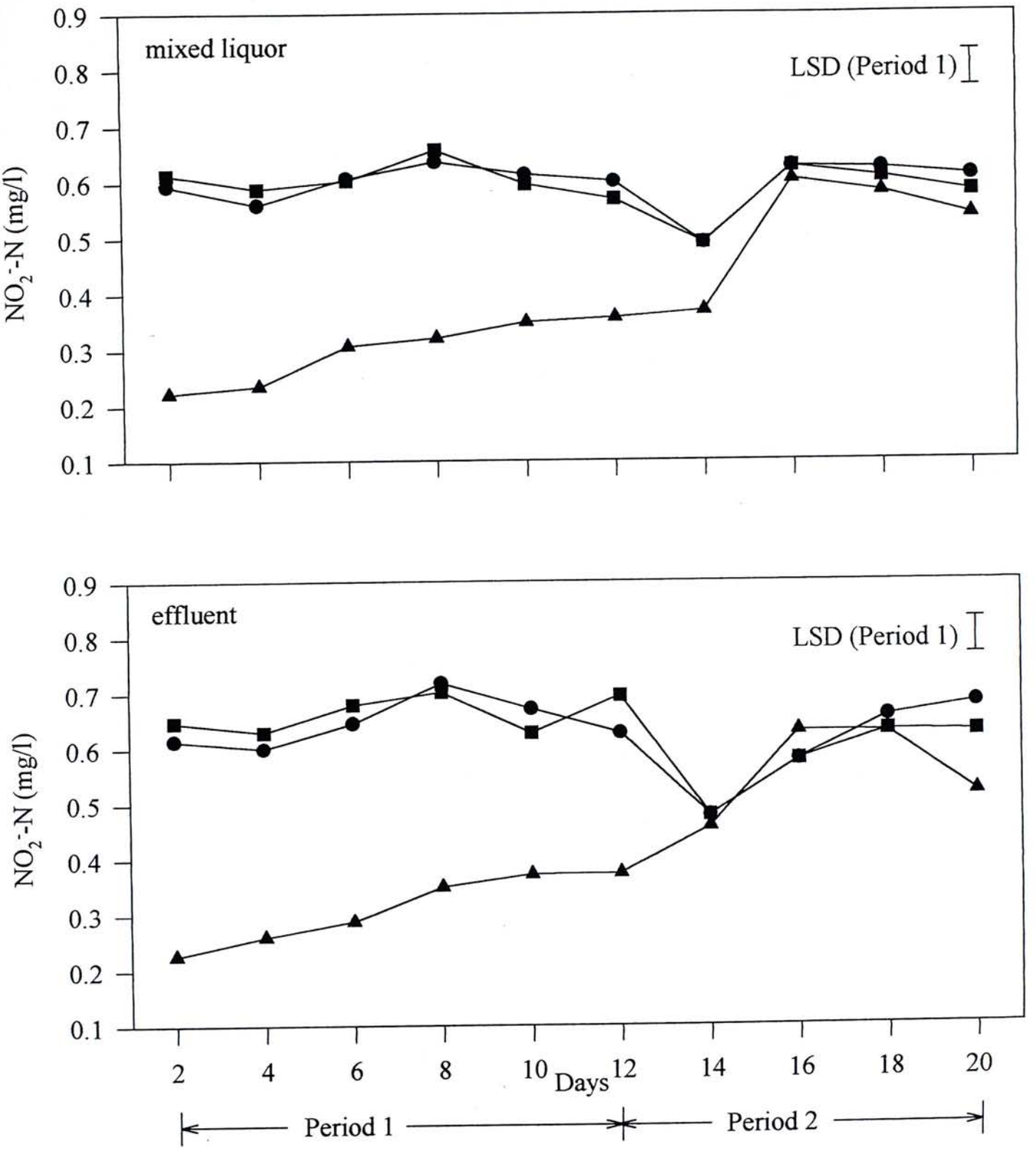


Fig. 4.11 Nitrite-N concentrations of mixed liquor and effluent from systems fed with (1) unspiked leachate containing 42.5 mg/L BOD (control) (●), (2) leachate spiked with methanol to 100 mg/L BOD (■) and (3) leachate spiked with methanol to 1000 mg/L BOD (▲) for 12 days (Period 1) and then with unspiked leachate for 8 days (Period 2). Values are means of three replicates measured every two days. Vertical bars denote LSD between different BOD level by the Tukey's Honestly Significant Difference Test during Period 1 at $P = 0.05$. No significant difference was found between different BOD levels by ANOVA during Period 2 at $P < 0.05$.

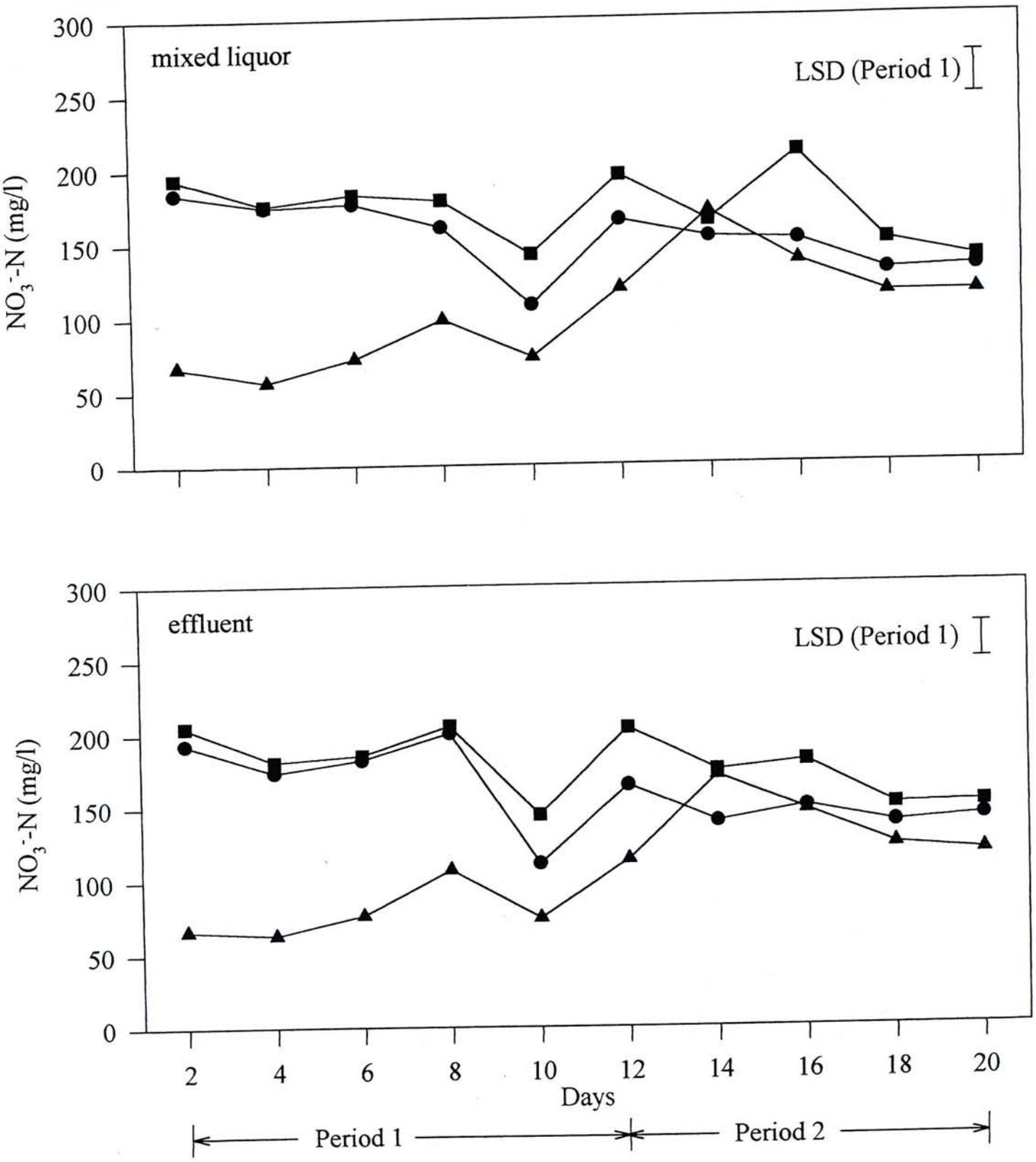


Fig. 4.12 Nitrate-N concentrations of mixed liquor and effluent from systems fed with (1) unspiked leachate containing 42.5 mg/L BOD (control) (●), (2) leachate spiked with methanol to 100 mg/L BOD (■) and (3) leachate spiked with methanol to 1000 mg/L BOD (▲) for 12 days (Period 1) and then with unspiked leachate for 8 days (Period 2). Values are means of three replicates measured every two days. Vertical bars denote LSD between different BOD level by the Tukey's Honestly Significant Difference Test during Period 1 at $P = 0.05$. No significant difference was found between different BOD levels by ANOVA during Period 2 at $P < 0.05$.

was unlikely that the activities of ammonia oxidizers were hindered by competition for ammonia with heterotrophic bacteria. Half-saturation coefficient of DO for *Nitrosomonas* is 0.15 - 2.0 mg/L O_2 (Environmental Protection Agency, 1975). Much higher DO level (6.0 - 7.4 mg/L) was maintained to avoid inhibition of nitrification at high organic loading rate. Lower nitrate concentration found in system was probably due to the removal of nitrite and nitrate by denitrification which also occurs at well-aerated condition (details discussed in Section 4.3.5). Excess organic carbon left over by heterotrophic bacteria was used by denitrifiers.

Increase of BOD did markedly raise the concentration of MLSS and MLVSS. During Period 1 when methanol-spiked leachate was fed into the system, MLSS and MLVSS of the system with influent containing 1000 mg/L BOD was about 3 times higher than those of the control (Fig. 4.13). Solids of system with 100 mg/L BOD was slightly higher than control during Period 1 although no significant difference ($P > 0.05$) was observed (Table 4.10). Concentrations of MLSS and MLVSS were more or less the same for Periods 1 and 2 as all solids were returned back to the aeration tank. If Period 2 is extended, there may be a decline of solid concentration due to the natural die-off of the heterotrophic bacteria which did not have organic carbon for growing.

Similar to solid concentration, the increase in BOD to 1000 mg/L also increased bacterial population. Populations of ammonia oxidizers and heterotrophic bacteria in mixed liquor was 3 - 10 times as high as those of the other two systems (Table 4.11). Likewise, population of nitrite oxidizers was about 1.2 - 2 times as high as the other two systems. Methanol added to influent provides organic carbon for the growth of heterotrophic bacteria. Heterotrophic bacteria provide a suitable matrix to

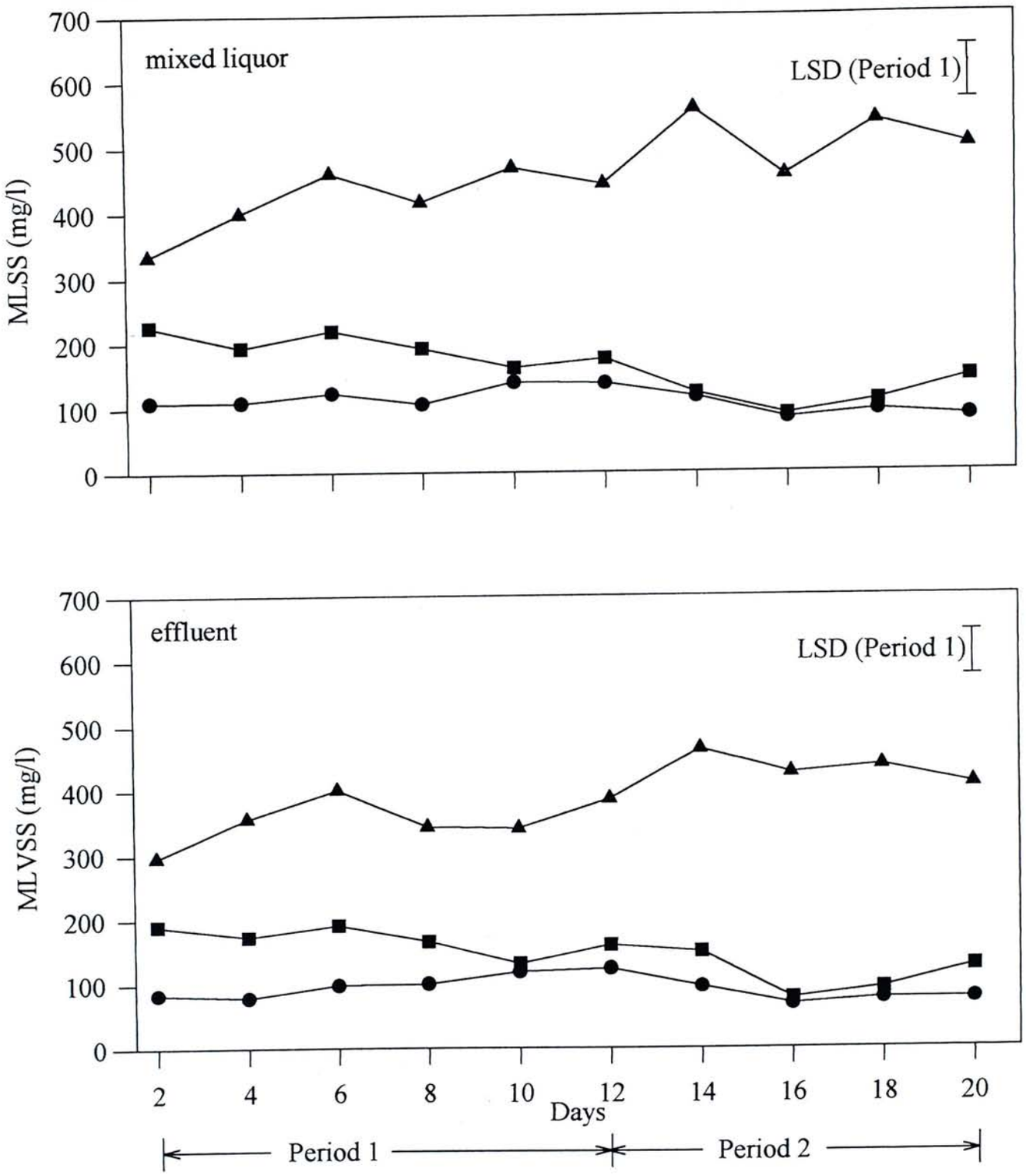


Fig. 4.13 MLSS and MLVSS of systems fed with (1) unspiked leachate containing 42.5 mg/L BOD (control) (●), (2) leachate spiked with methanol to 100 mg/L BOD (■) and (3) leachate spiked with methanol to 1000 mg/L BOD (▲) for 12 days (Period 1) and then with unspiked leachate for 8 days (Period 2). Values are means of three replicates measured every two days. Vertical bars denote LSD between different BOD level by the Tukey's Honestly Significant Difference Test at $P = 0.05$.

Table 4.11 Population of ammonia oxidizers, nitrite oxidizers and heterotrophic bacteria in mixed liquor of systems fed with (1) unspiked leachate containing 42.5 mg/L BOD (control), (2) leachate spiked with methanol to 100 mg/L BOD and (3) leachate spiked with methanol to 1000 mg/L BOD in Period 1. Values are means and standard deviations of three replicates measured on 6th and 12th day of the experiment.

	Ammonia oxidizers ($\times 10^5$ MPN/mL)	Nitrite oxidizers (MPN/mL)	Heterotrophic bacteria ($\times 10^6$ CFU/mL)
6th day			
control	0.32±0.15	16.5±2.0	0.25±0.13
100 mg/L BOD	0.56±0.33	24.0±0.0	0.25±0.01
1000 mg/L BOD	1.85±0.50	33.0±0.0	2.20±1.45
12th day			
control	0.18±0.07	24.0±0.0	0.77±0.23
100 mg/L BOD	0.45±0.32	26.7±5.5	0.83±0.07
1000 mg/L BOD	1.85±0.78	35.3±12.7	2.42±1.45
LSD	0.58	2.21	1.09

LSD denotes least significant difference ($P < 0.05$) in population due to difference in BOD levels by Tukey's Honestly Significant Difference Test.

form flocs which facilitate the retention of nitrifiers in the systems.

The higher ammonia removal rate of system with influent containing 1000 mg/L BOD (Table 4.12) is probably due to a combined effect of assimilation by hetertrophic bacteria and increased efficiency in nitrification due to higher nitrifier population. However, low concentrations of nitrite and nitrate could also be due to a decrease in their production by nitrification due to the inhibitory effects of toxic organic compounds (Robertson and Kuenen, 1992; Silverstein and Schroeder, 1983) or an increase of removal due to presence of surplus organic carbon. Although it is not known whether ammonia had been removed by nitrification or by assimilation, increase in BOD level to 1000 mg/L of the MYT leachate did improve the nitrogen removal efficiency. Once the system was fed with unspiked leachate in Period 2, percentage of ammonia removal decreased from 68.5% to 60.9% when comparing concentration in mixed liquor (Table 4.13). Nitrite and nitrate increased to level similar to the control.

On the other hand, systems with unspiked influent and influent containing 100 mg/L BOD showed similar concentrations of nitrite, nitrate, MLSS and MLVSS (Tables 4.10 and 4.13); ammonia removal (Table 4.12) in Periods 1 and 2. populations of ammonia oxidizers, nitrite oxidizers and heterotrophic bacteria in Period 1 (Table 4.11); Organic carbon level of 100 mg/L BOD was ineffective in improving biological nitrogen removal.

4.3.5 Inhibition of free ammonia and nitrous acid

Unionized ammonia (NH_3) and unionized nitrous acid (HNO_2) inhibit nitrifiers. *Nitrosomonas* and *Nitrobacter* were inhibited by 8.23 - 124 and 0.08 - 0.82

Table 4.12 Ammonia removal of systems fed with (1) unspiked leachate containing 42.5 mg/L BOD (control), (2) leachate spiked with methanol to 100 mg/L BOD and (3) leachate spiked with methanol to 1000 mg/L BOD for 12 days (Period 1) and then all systems fed with unspiked leachate for 8 days (Period 2). Values are means and standard deviations of three replicates measured every two days.

	Period 1		Period 2	
	Influent-mixed liquor	Influent-effluent	Influent-mixed liquor	Influent-effluent
NH_x-N removed (%)[a]				
control	65.5±2.9	63.0±2.0	62.9±4.0	62.5±2.8
100 mg/L BOD	62.8±3.6	60.8±3.1	57.5±4.6	58.7±8.7
1000 mg/L BOD	68.6±5.4	76.1±9.4	60.9±5.7	63.4±9.0
LSD	3.3	4.6	NS	NS
NH_x-N removed/MLSS (mg NH_x-N/mg MLSS/d)[b]				
control	2.37±0.90	2.29±0.87	3.18±1.16	3.17±1.18
100 mg/L BOD	1.46±0.23	1.41±0.22	2.20±0.60	2.24±0.69
1000 mg/L BOD	0.93±0.34	1.03±0.37	0.53±0.16	0.55±0.15
LSD	0.43	0.42	0.75	0.79
NH_x-N removed/MLVSS (mg NH_x-N/mg MLVSS/d)[c]				
control	2.77±1.03	2.68±1.00	3.78±1.28	3.18±1.16
100 mg/L BOD	1.70±0.31	1.65±0.29	2.46±0.81	2.20±0.60
1000 mg/L BOD	1.09±0.39	1.20±0.43	0.63±0.18	0.53±0.16
LSD	0.50	0.49	0.87	0.90

[a] NH_x-N removed (%)

$$= \frac{\text{influent conc. - mixed liquor or effluent conc.}}{\text{influent conc.}} \times 100\%$$

[b] NH_x-N removed/MLSS (mg NH_x-N/mg MLSS/day)

$$= \frac{(\text{influent conc. - mixed liquor or effluent conc.}) \times \text{flow rate}}{\text{MLSS conc.} \times \text{reactor volume}}$$

[c] NH_x-N removed/MLVSS (mg NH_x-N/mg MLVSS/day)

$$= \frac{(\text{influent conc. - mixed liquor or effluent conc.}) \times \text{flow rate}}{\text{MLVSS conc.} \times \text{reactor volume}}$$

LSD denotes least significant difference ($P < 0.05$) in removal rate due to difference in BOD by the Tukey's Honestly Significant Difference Test.

NS denotes no significant difference ($P > 0.05$) according to ANOVA.

Table 4.13 Quality of influent, mixed liquor and effluent in Period 2 (lasted for 8 days) from systems fed with unspiked leachate after feeding with (1) unspiked leachate containing 42.5 mg/L BOD (control), (2) leachate spiked with methanol to 100 mg/L BOD and (3) leachate spiked with methanol to 1000 mg/L BOD for 12 days (Period 1). Values are means and standard deviations of three replicates measured every two days for 8 days.

	Period 2			LSD[a]
	Control	100 mg/L BOD	1000 mg/L BOD	
NH_x-N (mg/L)				
influent	866±72	831±76	840±59	-
mixed liquor	319±28	351±25	327±41	NS
effluent	324±16	340±61	305±67	NS
LSD[b]	57	58	46	
NO_2^--N (mg/L)				
influent ($\times 10^{-3}$)	0.09±0.06	0.10±0.05	0.34±0.06	-
mixed liquor	0.60±0.03	0.58±0.10	0.56±0.05	NS
effluent	0.63±0.05	0.59±0.11	0.55±0.17	NS
LSD[b]	0.10	0.08	0.04	
NO_3^--N (mg/L)				
influent	0.23±0.12	0.49±0.07	0.50±0.10	-
mixed liquor	141±21	165±67	134±25	NS
effluent	142±9	164±40	140±23	NS
LSD[b]	20	45	13	
MLSS (mg/L)	95±32	116±32	518±141	82
MLVSS (mg/L)	79±24	108±42	433±110	69

[a] LSD denotes least significant difference ($P < 0.05$) in quality due to difference in BOD by the Tukey's Honestly Significant Difference Test.

[b] LSD denotes least significant difference ($P < 0.05$) between quality of influent, mixed liquor and effluent by the Tukey's Honestly Significant Difference Test.

NS denotes no significant difference ($P > 0.05$) according to ANOVA.

mg/L NH_3-N respectively (Anthonisen *et al.*, 1976). Both *Nitrosomonas* and *Nitrobacter* were inhibited by 0.07 - 0.85 mg/L HNO_2-N. Another study by Ford and coworkers (1980) found that inhibition of *Nitrosomonas* and *Nitrobacter* occurred when free ammonia concentration was 30 and 0.28 - 1.00 mg/L NH_3-N respectively. However, activity of *Nitrosomonas* could recover after being exposed to 70 mg/L NH_3-N.

$$NH_4^+ + OH^- \rightleftharpoons \underbrace{\boxed{NH_3 + H_2O}}_{\text{ammonia equilibrium}} \qquad \text{Eq. 4.5}$$

$$NH_4^+ + 1.5O_2 \longrightarrow H_2O + H^+ + \underbrace{\boxed{H^+ + NO_2^- \rightleftharpoons HNO_2}}_{\text{nitrous acid equilibrium}} \qquad \text{Eq. 4.6}$$

Concentrations of free ammonia and free nitrous acid in solution depend on concentration of ammonium/nitrite ion, pH and temperature and can be calculated by the following equation:

$$\text{Free ammonia (mg/L } NH_3\text{-N)} = \frac{NH_x\text{-N (mg /L)} \times 10^{pH}}{K_b/K_w + 10^{pH}} \qquad \text{Eq. 4.7}$$

K_b = the ionization constant of ammonia equilibrium (Eq. 4.5)
K_w = the ionization constant of water
$K_b/K_w = e^{6344/(273+T)}$
T = temperature in °C

$$\text{Free nitrous acid (mg/L } HNO_2\text{-N)} = \frac{NO_2^-\text{-N (mg/L)}}{K_a \times 10^{pH}} \qquad \text{Eq. 4.8}$$

K_a = the ionization constant of nitrous acid equilibrium (Eq. 4.6)
$= e^{-2300/(273+T)}$

From the above equation, it can be found that free ammonia inhibition is likely to occur at alkaline pH and higher temperature; while free nitrous acid inhibition is at acidic pH and lower temperature. The average pH before and after daily pH adjustment of the three experiments were 6.8 and 7.6 respectively. The temperature of system ranged from 24 - 27°C. The maximum free ammonia

concentration would occur at pH 7.61 and 27°C and maximum free nitrous acid concentration at pH 6.8 and 24°C. According to the calculated values, no inhibition of nitrifiers would occur due to free nitrous acid since the concentration was much lower than the inhibitory level (Table 4.14). However, free ammonia of the systems of present study was about 6 to 100 mg/L which was within the reported inhibitory range. Nitrifiers, especially *Nitrobacter*, would likely be inhibited.

No accumulation of nitrite was observed in all the experiments (all systems had nitrite concentration below 0.70 mg/L) (Tables 4.2, 4.7 and 4.10). The adverse conditions to *Nitrobacter* might disappear once the pH of the system was lowered and the effect of free ammonia eliminated. Acclimation of nitrifiers might also lower the degree of inhibition and restore nitrification activity (Anthonisen *et al.*, 1976).

A wide range of inhibiting levels of free ammonia to ammonia oxidizers was reported and the difference may due to the degree of acclimation. Ammonia-oxidizing bacteria with different sensitivities to free ammonia have been found (Suwa *et al.*, 1994). Ammonia oxidizers from sewage treatment plants and a laboratory culture, which had been acclimated in artificial wastewater containing organic nutrients, could only grow in medium with 19.9 mg NH_4^+-N/L but not that with 1000 mg NH_4^+-N/L. Other laboratory cultures, which had been acclimated in inorganic nutrients, could grow in both low- and high- ammonium media. Leachate used in the present study had high ammonia but low organic concentration. This could induce the development of ammonia-resistant nitrifiers.

4.3.6 Fate of ammonia

A decrease in ammonia concentration together with increases in

Table 4.14 Theoretical concentrations of free ammonia and free nitrous acid in aeration tank.

	NH_x-N conc. of aeration tank (mg/L)	NH_3-N conc. of aeration tank[a] (mg/L)	NO_2^--N conc. of aeration tank (mg/L)	HNO_2-N conc. of aeration tank[b] (mg/L)
Experiment 1 Effect of additional phosphate on rate of nitrification				
control	242	6.29	0.43	1.57×10^{-4}
5 mg/L PO_4^{3-}-P	235	6.11	0.52	1.90×10^{-4}
10 mg/L PO_4^{3-}-P	251	6.52	0.54	1.96×10^{-4}
Experiment 2 Effect of HRT on rate of nitrification				
1 day	431	11.2	0.43	1.57×10^{-4}
2 days	331	8.60	0.51	1.87×10^{-4}
4 days	322	8.37	0.50	1.84×10^{-4}
Experiment 3 Effect of additional organic carbon on rate of nitrification				
Period 1				
control	297	7.28	0.60	2.20×10^{-4}
100 mg/L BOD	354	9.20	0.61	2.21×10^{-4}
1000 mg/L BOD	280	7.72	0.30	1.09×10^{-4}
Period 2				
control	319	8.50	0.60	2.20×10^{-4}
100 mg/L BOD	351	9.12	0.58	2.11×10^{-4}
1000 mg/L BOD	327	8.29	0.56	2.06×10^{-4}

[a] Value calculated by Eq. 4.7. pH and temperature were 7.61 and 27°C respectively.

[b] Value calculated by Eq. 4.8. pH and temperature were 6.80 and 24°C respectively.

concentrations of nitrite and nitrate confirm that nitrification occurs. However, the total concentration of inorganic nitrogen of the mixed liquor and effluent was much lower than that of influent (Table 4.15). This suggests that processes other than nitrification are responsible for nitrogen removal. Possible routes of removal of inorganic nitrogen included:

(a) loss of ammonia to environment by air stripping

Since there is no parallel study on ammonia removal by air stripping, the actual amount of nitrogen lost through the this route is unknown. According to air stripping test conducted by Ford and co-workers (1980), less than 10% of ammoniacal-N was removed from sewage with NH_x-N concentration ranging from 119 - 206 mg/L when pH and temperature were less than 8.3 - 8.5 and 30°C respectively. Another study by Cheung (in press) showed that 10% ammoniacal-N was removed from landfill leachate at 20 - 23°C with initial NH_x-N concentration and pH of 705 mg/L and 7.5 respectively.

According to Eq. 4.7, free ammonia would exist at higher percentage when the temperature and pH are higher. Ammonia removed by air stripping increases under these condition. For the pH and temperature conditions of this study, which had temperature of 24 - 27°C and pH of 6.81 - 7.61, about 10 - 20% of NH_x-N could be lost through air stripping.

(b) conversion of nitrate to nitrogen gas

Though denitrification is an anaerobic process, it is widely found in well-aerated conditions, such as activated sludge tank, oxidation ditch, soil and shaking culture flask (Focht and Verstrate, 1977). Denitrification can occur simultaneously with nitrification because the existence of many anaerobic microsites, which are

Table 4.15 Total concentration of inorganic nitrogen (ammoniacal-N + nitrite-N + nitrate-N) of influent, mixed liquor and effluent. Values are the means of whole period of the experiments, i.e. 8 days for Period 2 of Experiment 3 and 12 days for the other experiments.

	Inorganic nitrogen (mg/L)		
	Influent	Mixed liquor	Effluent
Experiment 1 Effect of additional phosphate on rate of nitrification			
control	631±37	357±66	356±66
5 mg/L PO_4^{3-}-P	654±36	333±80	358±147
10 mg/L PO_4^{3-}-P	640±41	356±65	388±103
Experiment 2 Effect of HRT on rate of nitrification			
1 day	845±43	554±58	486±35
2 days	800±10	492±6	485±13
4 days	804±8	513±26	433±55
Experiment 3 Effect of additional organic carbon on rate of nitrification			
Period 1			
control	891±76	361±68	297±116
100 mg/L BOD	894±49	512±51	539±48
1000 mg/L BOD	861±62	460±36	489±30
Period 2			
control	840±60	462±40	445±62
100 mg/L BOD	831±76	517±70	504±85
1000 mg/L BOD	866±73	461±32	467±14

frequently too small to measure with an oxygen probe. The high oxygen consumption rate of nitrifiers facilitates the formation of these anaerobic microsites. The flocs of biomass provide another anaerobic microsite for denitrification.

(c) conversion of inorganic nitrogen to biomass

Ammonia oxidizers and nitrite oxidizers derive energy from oxidation of ammonia (Eqs. 4.1 and 4.2) and nitrite (Eqs. 4.3 and 4.4) and energy is utilized for the formation of biomass (Sharma and Ahlert, 1977).

Andreadakis (1993) found that cell synthesis was responsible for 13 - 17% of nitrogen removal in a suspended growth nitrification system. In a SBR system, 20 to 30% of nitrogen was removed by nitrogen assimilation (Silverstein and Schroeder, 1983).

4.4 CONCLUSIONS

Rate of nitrification, quality of effluent and population of microorganisms were similar irrespective of the phosphate concentrations in leachate. HRT of 2 days produced effluent of the highest quality in the shortest period of time. Increasing the HRT to 4 days increased the solids concentration but not the population of nitrifiers and the effluent quality. Based on ammonia removal per day, nitrifiers have the potential to treat influent with much higher ammonia concentration provided that toxic level of ammonia is not reached.

Increase of BOD of influent to 1000 mg/L by adding methanol can improve the nitrogen removal efficiency regarding the concentration of ammonia, nitrite and nitrate when compared with the system without or with low level (100 mg/L) carbon addition. Increase of ammonia assimilation by heterotrophic bacteria, improvement

of retention of nitrifiers and occurrence of denitrification were responsible to the better performance of the system. The high efficiency could only be maintained in the presence of high organic carbon. Efficiency decreased to level similar to control once the system was fed with unspiked leachate.

After the nitrification process, although ammonia concentration of the leachate was reduced, nitrate increased to very high level (> 100 mg/L). Methods which convert the nitrite and nitrate produced to non-toxic compounds should be considered.

5 DENITRIFICATION OF NITRIFIED LEACHATE

5.1 INTRODUCTION

For the complete treatment of an ammonia-rich wastewater, denitrification process is required to remove the oxidized-N produced at the nitrification stage. Otherwise, nitrate in the effluent will enrich the receiving water and lead to the occurrence of eutrophication. If the receiving water body is a source of drinking water, toxicity caused by nitrite and nitrate is of important concern. Nitrate is readily reduced to nitrite in mouth and digestive tract. Nitrite can react with amines and amides to form nitrosamines and nitrosoamides which are carcinogenic. Nitrite also reacts with hemoglobin to produce methemoglobin and may result in anoxia.

Denitrification can convert the environmentally undesirable oxidized-N to inert nitrogen gas that has no significant effect on the environment. Biological denitrification involves the microbial reduction of nitrate to nitrite and then to nitrogen gas (Eq. 5.1).

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2 \qquad \text{Eq. 5.1}$$

Unlike nitrification which is carried out by only few genera of bacteria, a wide range of bacteria, such as *Pseudomonas, Archromobacter, Micrococcus, Halobacterium, Spirillium and Thiobacillus*, are responsible for denitrification (Focht and Verstrate, 1977) and can be found in most natural environment. Most denitrifiers can reduce NO_3^- via NO_2^-, NO, N_2O and finally to N_2. However, some species may lack a particular key enzyme, e.g. NO_3^- reductase or N_2O reductase, and cannot reduce the specific nitrogen compound (Robertson and Kuenen, 1992). Denitrifiers can use either oxygen or nitrate as the electron acceptors in respiratory electron transport

chain for energy production. However, slightly less energy is generated when nitrate is used. Therefore, denitrifiers use oxygen preferentially, but denitrification occurs in anaerobic condition when nitrate is present. Unlike nitrifiers which are very sensitive to environmental conditions such as pH, temperature, unionized ammonia and nitrous acid, denitrifier can exist in wide range of conditions (Robertson and Kuenen, 1992; Environmental Protection Agency. 1993). Not all denitrifying bacteria can completely reduce nitrate to molecular nitrogen. A group of facultative anaerobic nitrate respiring bacteria utilizes nitrate as a terminal electron acceptor but cannot further reduce the nitrite to nitrogen gas (Focht and Verstrate, 1977).

During denitrification, organic compounds act as electron donors of the electron transport chain for energy production and carbon sources for biomass synthesis. In wastewater treatment plant, organics in wastewater and external carbon source (of which methanol is the most commonly used) are the major source of electron donors. Theoretically, denitrifying one gram of nitrate-N requires 2.86 g COD (Environmental Protection Agency, 1993). In actual environment, more organic compounds are required as portion of them is used for production of new cell mass.

Design of majority of conventional wastewater treatments have nitrification and denitrification processes exist exclusively, either in separated reactors or at different phases of treatment (sequencing batch reactor). Autotrophic nitrifiers require a relatively high oxygen concentration and low organic carbon. Preferable condition for denitrifiers is vice versa, i.e. low oxygen concentration and high organic carbon.

Sewage with 34.8 - 36.16 mg/L NH_x-N was treated by batch reactor with nitrification and denitrification processes (Tam *et al.*, 1992). After seven hours of

aeration, ammonia concentration decreased to about 2 mg/L and nitrate concentration increased to 30 mg/L. A subsequent 5-hour anaerobic period with additional carbon of 100 and 200 mg/L COD reduced nitrate to less than 6 mg/L.

Ammonia must be nitrified to nitrite or nitrate before being converted to nitrogen gas by denitrification. However, anoxic reactor may be installed before aerobic reactor, e.g. denitrification-nitrification system. The nitrified wastewater is then recycled back to the anoxic zone for denitrification. This configuration can prevent the problem of raising sludge due to the release of nitrogen gas in the clarifier. The downstream aerobic reactor also prevents the leakage of surplus carbon from anoxic reactor. However, total nitrogen removal cannot be achieved because effluent is discharged from aerobic reactor where nitrate is produced. A denitrification-nitrification system was used to treat a landfill leachate with ammonia concentration of 170 - 240 mg/L and BOD of 10 - 60 mg/L (Carley and Mavinic, 1991). Complete nitrogen removal was achieved in anoxic reactor when COD:NO_x-N ratio was between 5.9:1 to 9:1. As long as COD:NO_x-N ratio was lower than 20:1, all the carbon could be removed by the anoxic reactor. A full-scale denitrification-nitrification wastewater treatment plant was employed to treat wastewater from a chemical processing plant. The wastewater had BOD concentration of 1230 mg/L, total Kjeldahl nitrogen (TKN) of 190 mg/L, ammoniacal-N of 130 mg/L and oxidized-N of 175 mg/L (Sutton *et al.*, 1981). Very good efficiency of carbon and nitrogen removal was obtained at 20°C. Effluent produced had filtered COD of 20 mg/L, ammonia concentration less than 4 mg/L and nitrate concentration of 3.3 - 30.3 mg/L.

Most of the wastewater purification techniques are developed for domestic sewage treatment. Carbon removal is usually the primary concern. The properties of

leachate are different from those of sewage. Acetogenic leachate has higher organic and ammonia concentration than sewage; methanogenic leachate contains less biodegradable carbon but abundant ammonia. Techniques for sewage treatment cannot be applied directly to landfill leachate. MYT and PPV leachates have very high ammonia concentration. The nitrified leachate produced by nitrification had higher ammonia and nitrate concentrations than that of other studies. The denitrification efficiency of the nitrified leachate is unknown. In this experiment, leachate collected from Ma Yau Tong Central Landfill was treated by nitrification and then by an anoxic reactor with different hydraulic retention time (HRT) in order to assess the efficiency of nitrogen removal from this ammonia-rich wastewater. This can provide information on designing treatment system for landfill leachate by nitrification and denitrification processes.

5.2 Materials and Methods

5.2.1 Collection and analysis of landfill leachate

Landfill leachate was collected from the Ma Yau Tong Central (MYT) Landfill in July 1995. The collected leachate was filled into 20-liter plastic carboys and were kept at 4°C before experiment. pH, dissolved oxygen (DO), electrical conductivity (EC), salinity, total solids (TS), alkalinity, chemical oxygen demand (COD), biochemical oxygen demand (BOD), total Kjeldahl nitrogen (TKN), ammoniacal-N (NH_x-N), nitrite-N (NO_2^--N), nitrate-N (NO_3^--N), total Kjeldahl phosphorus (TKP) and orthophosphate-P (PO_4^{3-}-P). Procedures and methods involved were identical to that described in Section 2.2.3 and 3.2.2.

5.2.2 Set-up of treatment system

Bench scale, continuous unit was used for the test. Leachate was firstly fed into the nitrification system as described in Section 4.2.2. No phosphate and carbon addition was practiced. HRT was maintained at 2 days with temperature controlled at 25±2°C. Settled nitrified leachate was filled into 20-liter carboy and was kept at 4°C before the denitrification experiment. Composition of the nitrified leachate was determined. Parameters determined were identical to that of the raw leachate. Methods involved followed those described in Section 2.2.3 and 3.2.2.

The anoxic system (Fig. 5.1) was an one-liter plastic container with 950 mL mixed liquor and another one-liter plastic container as the clarifier. Leachate was fed into the anoxic reactor at the bottom and allowed to flow out at the overflow outlet. The mixed liquor in the anoxic system was stirred at speed just high enough to keep solids in suspension by means of a bottom magnetic stirrer. The system was started by slowly feeding nitrified leachate to anoxic reactor which contained returned sludge from the Shatin Sewage Treatment Plant. Hydraulic retention time (HRT) of systems was 8 days when setting-up but decreased at a rate of 0.5 days per day to the specific HRT at the start of experiment. HRT of 1, 2 and 4 days were chosen for experiment and there were three replicates for each treatment. The systems were maintained at the specified flow rate for at least two weeks prior to sampling. Temperature was controlled at 25±2°C. DO of anoxic reactors were less than 2 mg/L. Influent into the anoxic system, mixed liquor in the anoxic reactor and effluent from the clarifier were sampled every two days for a period of eight days. Concentrations of ammoniacal-N, nitrite-N and nitrate-N were measured by phenate method, sulfamilamide method and brucine method respectively. MLSS and MLVSS of the reactors were also determined.

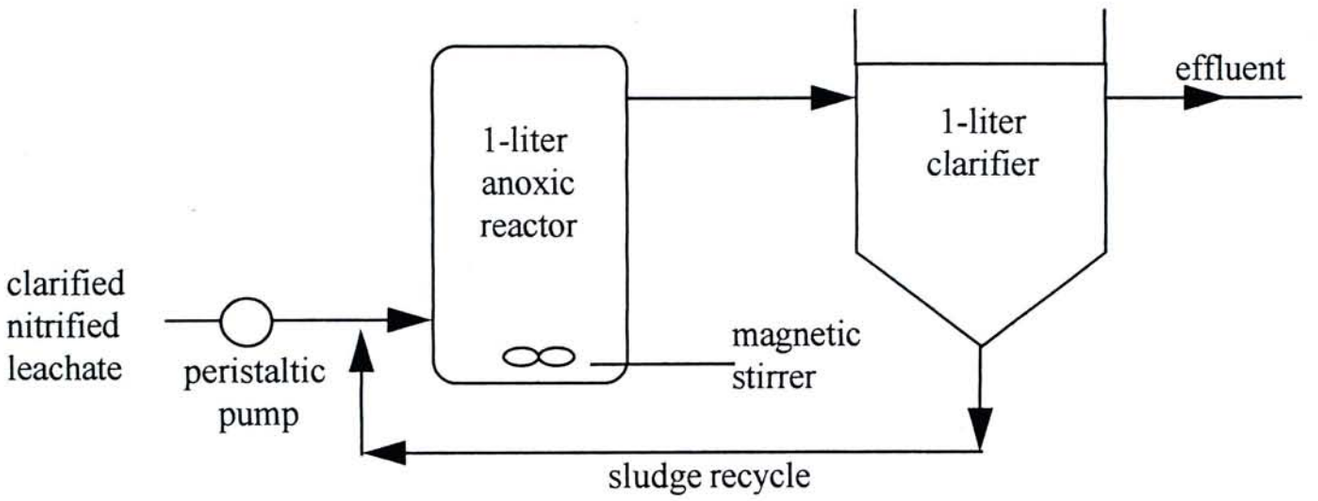


Fig. 5.1 The experimental setup for denitrification study.

5.2.3 Statistical analysis

Composition between raw and nitrified leachates were compared by t-test at a significant level of 0.05. The difference in leachate quality among influent, mixed liquor and effluent of the denitrification system and the difference in quality between mixed liquor and effluent due to HRT were tested by nested Analysis of Variance (ANOVA) with day as nested factor at $P < 0.05$. Least Significant Difference (LSD) was calculated by the Tukey's Honestly Significant Difference Test ($P < 0.05$) when significant difference was detected by ANOVA. All statistical analyses were performed by means of SPSS (Statistical Package for Social Science) for Windows Release 6.0 of SPSS Inc.

5.3 Results and Discussion

5.3.1 Performance of nitrification system

No phosphate was added and HRT of two days were chosen for the nitrification system according to the results of Chapter 4. No organic carbon was added although this could increase ammonia removal efficiency (Section 4.3.4). This is because surplus carbon in the nitrified leachate may affect the result of the denitrification experiment and increase in ammonia removal may partly be due to increased heterotrophic uptake instead of nitrification.

Composition between raw leachate and nitrified leachate is shown in Table 5.1. After passing through the nitrification system, pH, total solids (TS) and alkalinity of the leachate were greatly reduced. The acid-producing nitrification process resulted in decrease of alkalinity for more than 90%; pH of the nitrified leachate was lowered to 6.12. Sedimentation removed most of the solids in the leachate.

Table 5.1 Characteristics of raw and nitrified leachates used for denitrification experiment. Leachate was collected from Ma Yau Tong Central Landfill. Values are means and standard derivations of four replicates. Differences between raw and nitrified leachates were tested by Student's t-test.

Parameters	Raw leachate	Nitrified leachate	Remarks
pH	7.17±0.30	6.12±0.16	$P < 0.001$
DO (mg/L)	5.23±0.26	-	-
EC (μS/cm)	3830±10	4380±772	NS
Salinity ($^{o}/_{oo}$)	3.00±0.00	3.13±1.03	NS
Total solids (mg/L)	13600±2610	2150±431	$P < 0.001$
Alkalinity (mg/L)	2810±17	149±60	$P < 0.001$
COD (mg/L)	277±15	751±48	$P < 0.001$
BOD (mg/L)	25.1±0.7	20.5±2.6	$P < 0.05$
BOD/COD	0.09	0.03	$P < 0.05$
TKN (mg/L)	439±3	198±15	$P < 0.001$
NH_x-N (mg/L)	349±4	158±16	$P < 0.001$
NO_2^--N (mg/L)	<0.001	0.34±0.03	$P < 0.001$
NO_3^--N (mg/L)	0.04±0.01	128±9	$P < 0.001$
TKP (mg/L)	0.34±0.01	0.50±0.04	$P < 0.001$
PO_4^{3-}-P (mg/L)	0.22±0.06	0.13±0.02	$P < 0.01$

NS denotes no significant difference ($P < 0.05$) by Student's t-test.

COD and TKP of nitrified leachate increased significantly, although BOD and PO_4^{3-}-P levels were lower than that of raw leachate. These are probably attributed to the poor settleability of the nitrified effluent with some of the unsettled biomass washed out with the effluent.

About 55% ammonia was removed by the aerobic reactor. This efficiency was similar to that obtained in Chapter 4 (Table 4.8). Concentrations of nitrite and nitrate of nitrified leachate were increased significantly ($P < 0.05$). COD:NO_x-N ratio of the nitrified leachate increased to 5.87:1. This value was close to or higher than optimum ratio of 2.5:1 (Skrinde and Bhagat, 1982) and 5 - 5.5:1 (Manoharan, 1989) but lower than that reported by Carley and Mavinic (1991) which was 5.9:1 to 9:1. If denitrification can carry out effectively without external carbon addition, this can reduce the operation cost and also prevent elevated organic level in the effluent. One disadvantage of high COD:NO_x^--N ratio is that it favors the dissimilatory nitrate reduction by nitrate-respiring bacteria. Nitrate is only converted to nitrite but not further reduced to nitrogen gas (Focht and Verstrate, 1977; Robertson and Kuenen, 1992). Instead, nitrite may further be reduced to ammonia by nitrate-respiring bacteria for dumping excess reducing power. *Escherichia coli* and *Bacillus licheniformis* were found to carry out this kind of nitrate reduction (Robertson and Kuenen, 1992). Instead of adding external carbon source, denitrification can be carried out using organic compounds in the nitrified leachate. The idea of enhancing denitrification by endogenous organic materials has been proposed (Jones *et al.*, 1990; Silverstein and Schroeher, 1983). Therefore, no carbon addition was practiced in the anoxic reactor in the denitrification study.

5.3.2 Performance of denitrification system

Denitrification process had little effect on ammonia concentration. For the system with hydraulic retention time of 1 and 2 days, no significant change in concentration was found between influent, mixed liquor and effluent (Table 5.2). When HRT of the system was increased to 4 days, ammonia concentration was reduced for less than 7% (Fig. 5.2).

Similarly, denitrification had little effect on nitrite concentration. No significant difference between influent, mixed liquor or effluent was observed (Table 5.2 and Fig. 5.3). Nitrite is one of the products of denitrification process (Eq. 5.1). The rate of formation and removal may be similar so that nitrite concentration did not change significantly. Nitrate concentration of the system in the present study was high (> 80 mg/L) such a nitrate concentration was found to induce nitrite accumulation (Focht and Verstrate, 1977). However, accumulation of nitrite was not observed in this study.

Volume of the nitrified leachate and amount of nitrate entering each system with different HRT. Therefore, both the percentage of nitrate removal and total nitrate removed each day were compared (Table 5.3). Generally, total nitrate removed each day in system with shorter HRT was significantly higher than that with longer HRT. Higher nitrate loading rate increased the rate of nitrate removal by denitrification. Percentage of nitrate removal of system with HRT of 4 days was higher than that of 2 days which was in turn higher than that of 1 day. Longer retention time allowed more oxidized nitrogen to be reduced to nitrogen gas before the nitrified leachate flushed out of the system. However, nitrate removal efficiency of all the three systems was unsatisfactory; only about 10 to 30% of nitrate was removed and more than 70 mg/L nitrate was left in the effluent (Fig. 5.4). Nitrate

Table 5.2 Quality of influent, mixed liquor and effluent from denitrification system fed with nitrified leachate. Systems had HRT of 1, 2 and 4 days. Values shown are means of three replicates measured every 2 days for 8 days.

	HRT			LSD[a]
	1 day	2 days	4 days	
NH_x-N (mg/L)				
influent	158±16	158±16	158±16	
mixed liquor	155±15	154±14	146±13	14
effluent	152±13	148±11	142±12	NS
LSD[b]	NS	NS	13	
NO_2^--N (mg/L)				
influent	0.34±0.03	0.34±0.03	0.34±0.03	
mixed liquor	0.34±0.02	0.34±0.01	0.32±0.04	NS
effluent	0.35±0.01	0.37±0.07	0.32±0.04	0.05
LSD[b]	NS	NS	NS	
NO_3^--N (mg/L)				
influent	128±9	128±9	128±9	
mixed liquor	113±7	107±2	83.1±9.0	7
effluent	103±6	93.6±4.8	77.7±5.0	5
LSD[b]	7	5.8	7.3	
MLSS (mg/L)	272±33	261±44	258±28	NS
MLVSS (mg/L)	230±24	201±32	210±26	NS

[a] LSD denotes least significant difference ($P < 0.05$) in quality due to differences in HRT by Tukey's Honestly Significant Difference Test.

[b] LSD denotes least significant difference ($P < 0.05$) between quality of influent, mixed liquor and effluent by Tukey's Honestly Significant Difference Test.

NS denotes no significant difference ($P > 0.05$) according to ANOVA

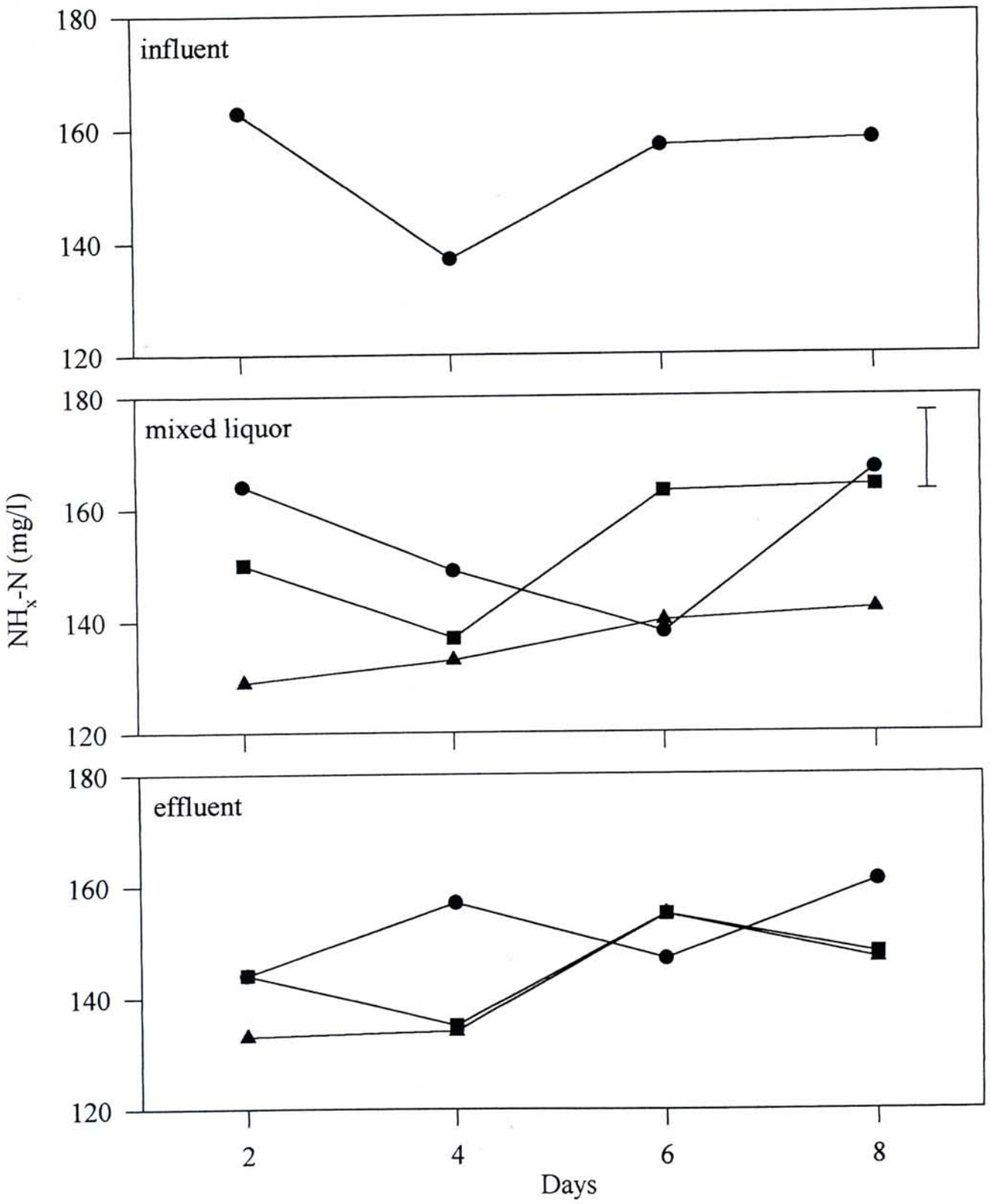


Fig. 5.2 Ammoniacal-N concentrations of influent, mixed liquor and effluent from denitrification systems with HRT of 1 (●), 2 (■) and 4 (▲) days. Values are means of three replicates measured every two days for 8 days. Vertical bar denotes LSD of mixed liquor quality between different HRT by the Tukey's Honestly Significant Difference test at $P = 0.05$. No significant difference was found between effluent quality by ANOVA at $P < 0.05$.

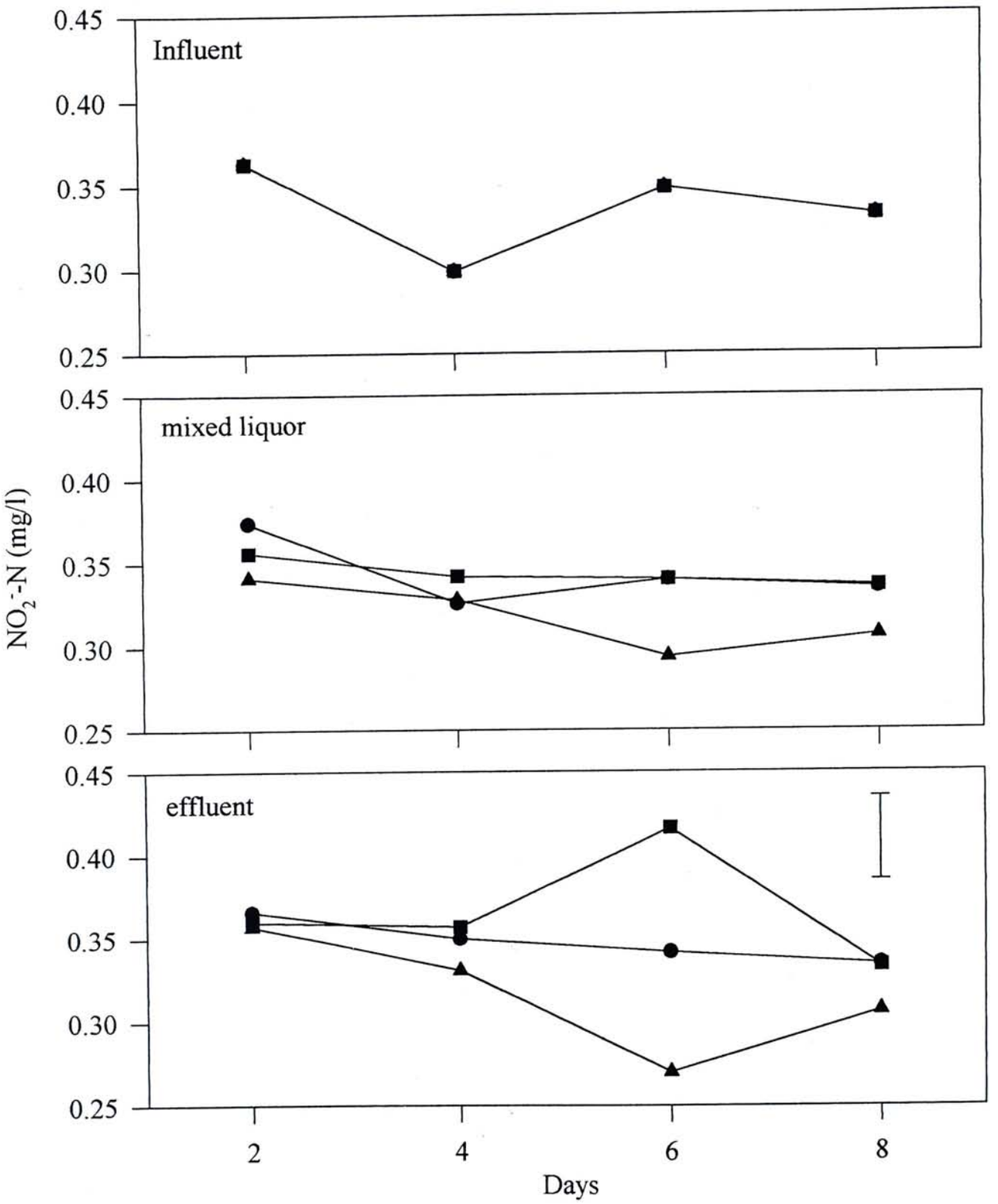


Fig. 5.3 Nitrite-N concentrations of mixed liquor and effluent from denitrification systems with HRT of 1 (●), 2 (■) and 4 (▲) days. Values are means of three replicates measured every two days for 8 days. No significant difference of mixed liquor quality was found between different HRT by ANOVA at $P < 0.05$. Vertical bar denotes LSD between effluent quality by the Tukey's Honestly Significant Difference test $P = 0.05$.

Table 5.3 Nitrate removal of denitrification system fed with nitrified leachate. Systems had HRT of 1, 2 and 4 days. Values shown are means of three replicates measured every 2 days for 8 days.

	Influent-mixed liquor	Influent-effluent
NO_3^--N removed (%)[a]		
HRT = 1 day	13.1±5.1	22.4±6.6
HRT = 2 days	21.9±9.6	31.4±9.9
HRT = 4 days	36.7±6.4	39.3±3.6
LSD	6.0	10
Total NO_3^--N removed (mg/d)[b]		
HRT = 1 day	15.7±6.3	25.2±7.2
HRT = 2 days	10.8±3.7	17.4±5.9
HRT = 4 days	11.3±3.1	12.7±2.2
LSD	4.6	5.5
NO_3^--N removed/MLSS (mg NO_3^--N/mg MLSS/d)[c]		
HRT = 1 day	0.05±0.03	0.09±0.03
HRT = 2 days	0.04±0.02	0.05±0.02
HRT = 4 days	0.05±0.02	0.06±0.02
LSD	NS	0.02
NO_3^--N removed/MLVSS (mg NO_3^--N/mg MLVSS/d)[d]		
HRT = 1 day	0.07±0.03	0.11±0.03
HRT = 2 days	0.05±0.02	0.08±0.03
HRT = 4 days	0.05±0.02	0.06±0.02
LSD[a]	NS	0.03

[a] NO_3^--N removed (%)

$$= \frac{\text{influent conc. - mixed liquor or effluent conc.}}{\text{influent conc.}} \times 100\%$$

[b] Total NO_3^--N removed (mg/d)

= (influent conc.- mixed liquor or effluent conc.) × flow rate

[c] NO_3^--N removed/MLSS (mg NO_3^--N/mg MLSS/d)

$$= \frac{\text{(influent conc. - mixed liquor or effluent conc.)} \times \text{flow rate}}{\text{MLSS conc.} \times \text{reactor volume}}$$

[d] NO_3^--N removed/MLVSS (mg NO_3^--N/mg MLSS/d)

$$= \frac{\text{(influent conc. - mixed liquor or effluent conc.)} \times \text{flow rate}}{\text{MLVSS conc.} \times \text{reactor volume}}$$

LSD denotes least significant difference ($P < 0.05$) in removal rate due to differences in HRT by Tukey's Honestly Significant Difference Test.

NS denotes no significant difference ($P > 0.05$) according to ANOVA.

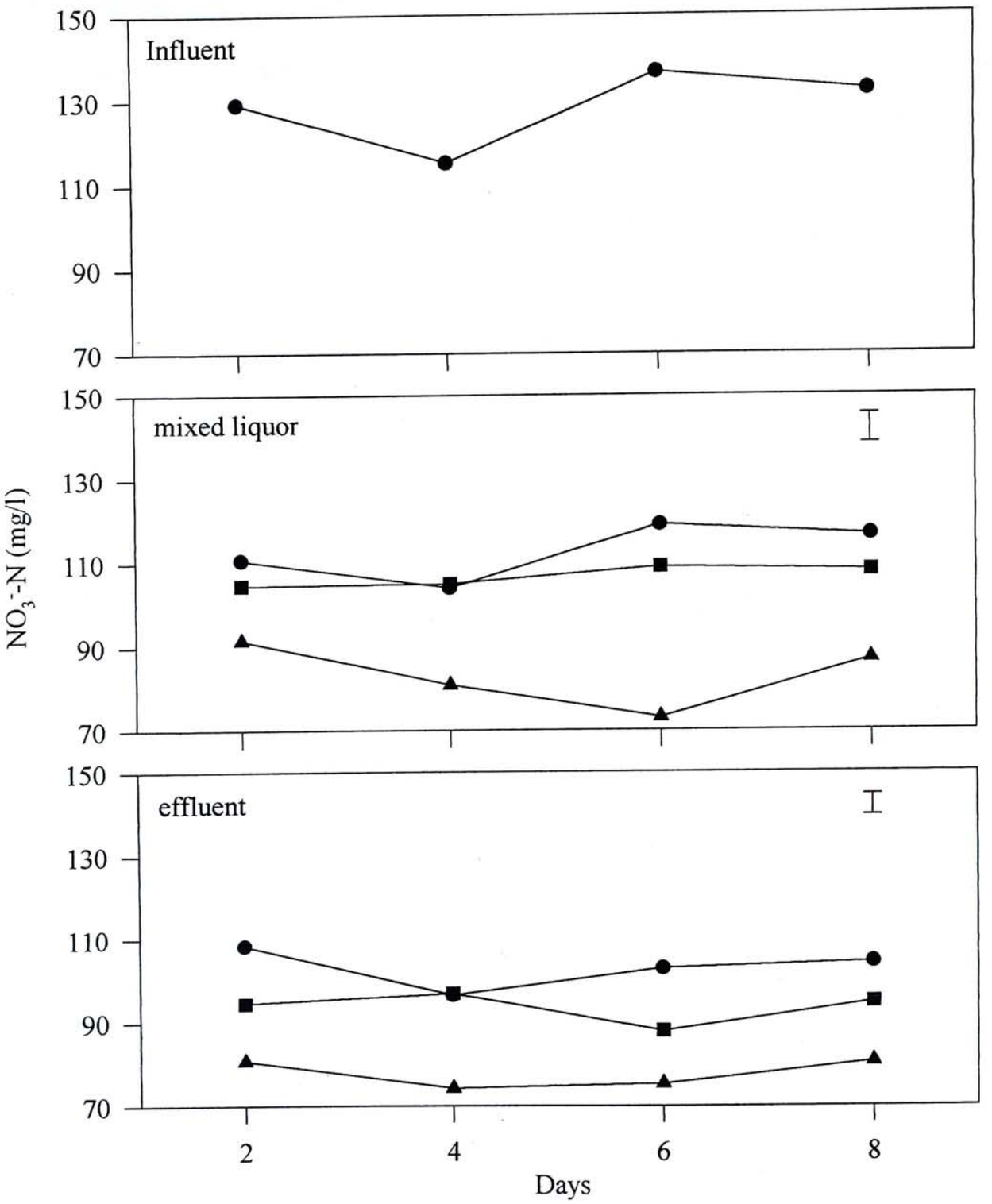


Fig. 5.4 Nitrate-N concentrations of influent, mixed liquor and effluent from denitrification systems with HRT of 1 (●), 2 (■) and 4 (▲) days. Values are means of three replicates measured every two days for 8 days. Vertical bars denote LSD between HRT by the Tukey's Honestly Significant Difference test $P = 0.05$.

concentration of effluent of all systems were significantly lower than that of mixed liquor. Denitrification process continued in the clarifier which was also anoxic. When denitrification is allowed to proceed, clarifier may suffer from floating sludge problem. This was, however, not observed in this study which might be due to low biomass concentration of the system.

5.3.3 Improvement of treatment efficiency

In this study, rates of nitrification and denitrification were not high enough to produce effluent with acceptable quality. The highest ammonia removal efficiency of the nitrification system was 65.5% whilst the highest nitrate removal efficiency of the denitrification system was 39.3% when there was no carbon addition. High concentration of ammonia and nitrate were still found in the effluent. One of the problem is the solid concentration (MLSS and MLVSS) of the present study was low when compared with other studies (Table 5.4). Without additional external carbon source, MLSS concentration of the nitrification and denitrification systems were 122 - 324 and 201 - 230 mg/L respectively and were about 10 folds lower than other study. Number of nitrifiers and denitrifiers in a system is closely related to the efficiencies of ammonia and nitrate removal (Takamizawa *et al.*, 1993). Although all of the settled sludge was returned to the aeration or anoxic reactor, it is not easy to maintain a high biomass concentration in a suspended growth system with low organic strength influent. Measures should be taken to retain the nitrifiers/denitrifiers and to prevent loss of microorganisms with the effluent.

For the nitrification system, low biomass concentration was due to a lack of

Table 5.4 Solid concentration of suspended growth nitrification and denitrification systems

System	Solid concentration	Reference
Aeration tank, batch scale	800-6000 mg/L MLVSS	Wild *et al.*, 1971
Nitrification-denitrification reactor, bench scale	2877-3197 mg/L MLSS at the beginning of cycle	Tam *et al.*, 1992
Sequencing batch reactor (SBR)	3994 - 6174 mg/L MLVSS at the end of aeration 4391-5458 mg/L MLVSS at the end of anoxic	Silverstein and Schroeher, 1983
Denitrification-nitrification reactor, bench scale	1500-6200 mg/L MLVSS	Carley and Mavinic, 1991
	927-1550 mg/L MLVSS	Andreadakis, 1993

heterotrophic bacteria which were essential to floc formation. Nitrifiers not settled in the clarifier were lost with the effluent. As shown in Section 4.3.3, increase of readily available carbon in the reactor can increase the rate of nitrogen removal. Therefore, when organic carbon is not high enough that heterotrophic bacteria outnumbered autotrophic nitrifiers, organic carbon has beneficial effect on ammonia removal. Beneficial effect of organic carbon on the rate of nitrification was also observed in another study (Fang *et al.*, 1993); addition of carbohydrates and proteins resulted in increase of nitrification efficiency of a rotating biological contactor (RBC). Although it was stated that carbohydrates and proteins acted as nutrients which stimulated the growth of nitrifiers, as nitrifiers are autotroph, it was more likely that supplement of organic carbon enhanced growth of heterotrophic bacteria which prevented the loss of nitrifiers.

For the denitrification system, a lack of readily-available carbon was probably the reason of low biomass concentration and thus low efficiency of nitrate removal. C:N ratio should not be used as the only index to determine treatability. Sewage from different sources have different rate of denitrification even they have similar C:N ratio (Andreadakis, 1993). This reflects that wastewater of similar C:N ratio may have different rate of denitrification because of different forms of carbon present in the wastewater. Moreover, nitrogen gas produced at denitrification process also affects the sedimentation of sludge in clarifier.

Poor sludge settleability were found in nitrification and denitrification system. Various methods have been tested by different scientists to maintain enough nitrifying and denitrifying microorganisms in the treatment tank. It is possible to provide a surface for the attachment of nitrifiers and/or denitrifiers. RBC and powdered

activated carbon treatment (PACT) seem to fulfill the requirement of leachate treatment process. Both these systems would not suffer from clogging problem caused by high calcium and iron salts of leachate. In PACT system, activated carbon can facilitate the removal of refractory carbon in leachate (Mcshane *et al.*, 1988; Ying *et al.*, 1987). Denitrification-nitrification system which had upstream anoxic reactor and downstream aerobic reactor was found to produce sludge with better settleability (Andreadakis, 1993). Immobilization of nitrifying and/or denitrifying bacteria in gel matrix such as calcium alginate and carrageenan has also been proposed (Robertson and Kuenen, 1992). The bacteria-containing gel was then placed back into the treatment system for removal of nitrogen compounds.

5.4 CONCLUSIONS

A low organic strength and ammonia rich landfill leachate was treated by a bench scale nitrification and denitrification system. Nitrification system with HRT of 2 days without phosphate and carbon amendment removed 55% of the ammonia. Nitrate accounted for 67% of ammonia removal. Concentration of COD was increased significantly. COD:NO_3^--N ratio increased to 5.87:1 which was within the reported optimum ratio for denitrification. Efficiency of denitrification system in treating the nitrified leachate was tested without addition of external organic carbon. However, nitrate removal efficiency was only about 10 - 30%. System with longer HRT had higher nitrate removal efficiency.

Effluent produced from this nitrification and denitrification system had more than 140 mg/L ammonia and 70 mg/L nitrate. Low biomass concentration may be responsible for the incomplete nitrogen removal. Sludge produced from both

nitrification and denitrification had poor settleability. Modification of the system is required to improve the efficiency of treating low organic strength leachate.

6 GENERAL CONCLUSIONS

Landfilling is the major solid waste disposal method in Hong Kong. Including both closed and operating landfills, there are totally 16 landfills in the territory. Landfill leachate is one of the major environmental problems caused by landfilling. The wet and hot weather in Hong Kong enhances the degradation of waste in landfill and produce a large volume of leachate. Leachate from the closed Ma Yau Tong Central Landfill (MYT) showed a characteristic of methanogenic leachate. Characteristic of leachate from the operating Pillar Point Valley Landfill (PPV) changed from slightly acetogenic at the start of the sampling period to methanogenic leachate at the end of the sampling period. Readily degradable compounds such as carbohydrates and proteins represented a small portion of the organics in leachate; refractory organic compounds accounted for most of the organic content. Ammonia was another polluting agent which existed in high concentration. Its toxicity and the possibility of inducing oxygen depletion and eutrophication of the receiving water make ammonia the primary component that must be removed before discharge. On the other hand, phosphorus and heavy metals which are the major polluting components in domestic sewage were present at low concentration in the leachates.

When biological toxicity test is employed as complementary parameters of chemical analysis for leachate characterization, toxicity assay using animals system is more suitable. MYT and PPV leachates caused acute toxicity to two animals used. 48-h LC50 of *Moina macrocopa* and 24- to 72-h LC50 of *Branchydanio rerio* were less than 20% and 10% of the leachates. Sensitivity of the Microtox test to the leachates was low. Among the four tested systems, *Chlorella pyrenoidosa* was the most tolerance to the leachates.

In present study, only gross organic contents were determined. Most of them is refractory to biological degradation. Individual compounds which were accounted for the refractory fraction were not determined. In addition to humic and fulvic acids formed in degradation by microorganisms, those of refractory nature are probably synthetic organics originated from the wastes buried in landfill. They may cause serious environmental problem even at low concentration. In future studies, concentrations of individual organic compounds, especially those in the list of organic priority pollutants (Hallbourg *et al.*, 1992; Oman and Hynning, 1994), in leachate from local landfills should be determined. Mutageneticity and chronic toxicity of leachate are also valuable for further investigation.

The temporal variability in chemical properties of leachates is a problem for treatment plant operation. Highly variable parameters were similar even for leachates from landfills of different age. Chemical oxygen demand (COD) and total solids (TS) exhibited high variation. These will affect treatment plant to a greater extent as they existed at higher concentration and are the key pollutants that must be removed. Good correlation of TS concentration and cumulative rainfall was found in both landfills, suggesting that rainfall record can be used for predicting leachate TS concentration. However, correlation of COD and rainfall was poor in the operating landfill.

Due to its high concentration and potential effect on environment, ammonia is the primary pollutant that must be removed from leachate. Nitrification and denitrification had the advantage over the other removal method since it can ultimately convert the polluting nitrogen compounds to an inert nitrogen gas. Although low phosphate level in leachate was found not to be the limiting factor of

nitrification, suspended growth system had difficulty in maintaining high solids concentration when treating low organic strength leachate. Though increase of hydraulic retention time could improve the treatment efficiency of nitrification system, degree of improvement decreased when limit was reached. Lower rate of nitrification was found for system with lower ammonia loading rate (long hydraulic retention time). Addition of organic carbon seemed to be a effective way to retain nitrifiers in suspended growth nitrification system and to improve ammonia removal. Even high ammonia and nitrate concentrations were present in mixed liquor, toxicity effects of free ammonia and nitrous acid to nitrifiers were not observed. Proper control of pH (Anthonisen *et al.*, 1976) and sufficient period of acclimation (Takahashi *et al.*, 1992) can protect sensitive nitrifiers.

Although nitrification significantly increased the organic content of the leachate, those organic compounds were not readily utilized by denitrifiers. Similar to nitrification system, the suspended growth denitrification system had problem in retaining the microorganisms in reactor and enhancing the efficiency in nitrate removal.

In order to treat ammonia-rich leachate, further work of nitrification and denitrification system with better biomass retention is needed. Attached growth system (Hosmi *et al.*, 1991; Meaney and Strickland, 1994; Spengel and Dzmbak, 1991), fludized bed system (Ying *et al.*, 1987) and denitrification-nitrification system (Andreadakis, 1993; Sutton *et al.*, 1981) are the potential candidates. Immobilized nitrifier and denitrifiers in gel matrix (Robertson and Kuenen, 1992) is also a possible way to improve biomass retention.

In addition to ammonia, COD was also present in very high concentration in

the leachates. Although this does not result in leachate with high oxygen demand, if chemical analysis reveals the present of high concentration of synthetic compounds or mutagenicity test reveals the present of hazardous compounds, treatment method aimed at refractory organic compounds is required. Precipitation/coagulation/ flocculation can only remove 20 - 40% COD even high dosage of chemical is employed (Boyle *et al.*, 1974; Chian and DeWalle, 1976; Diamadopoulos, 1994; Kinman and Nutini, 1992; Knox, 1983). Activated carbon adsorption is not effective in removal polar compounds and compounds with MW > 50,000. These hinder the use of either method as the single treatment for removing of refractory organics in a leachate. On the other hand, oxidation can increase the degradability of organic compounds (Kinman and Kutini, 1992). This allow the use of oxidation as a pretreatment step for biological treatment.

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APPENDIX 1 MEDIA FOR ENUMERATION OF HETEROTROPHIC BACTERIA, FUNGI, CARBOHYDRATE-UTILIZING BACTERIA, PROTEIN-UTILIZING BACTERIA AND LIPID-UTILIZING BACTERIA

1. Plate count agar (American Public Health Association, 1992)

Composition (per liter)

Agar	20.0 g
Typtone	5.0 g
Yeast extract	2.5 g
Glucose	1.0 g
pH	7.0 ± 0.2

Preparation of agar

Add components to distilled water and bring volume to one liter. Mix thoroughly. Generally heat and bring to boiling. Autoclave for 15 min at 121°C. Pour into sterilized petri dishes.

2. Neopeptone-glucose-rose bengal aureomycin agar (APHA, 1992)

Composition (per liter)

Agar	20.0 g
Neopeptone	5.0 g
Glucose	10.0 g
Rose bengal solution (1%)	3.5 mL
Tetracycline solution (1 g/150 mL)	5.0 mL
pH	6.5 ± 0.2

Preparation of agar

Add components, except tetracycline solution, to distilled water and bring volume to one liter. Mix thoroughly. Generally heat and bring to boil. Autoclaved for 15 min at 121°C. Cool to about 45°C. Sterilize tetracycline solution by filtration. Aseptically add 5 mL sterilized tetracycline solution to agar mixture. Pour into sterilized petri dishes.

3. Skim milk agar (Ronald, 1992)

Composition (per liter)

Agar	20.0 g
Casein	5.0 g
Yeast extract	2.5 g
Glucose	1.0 g
Skim milk solution	100 mL
pH	7.0 ± 0.1

Preparation of agar

Add components, except skim milk solution, to distilled water and bring volume to one liter. Mix thoroughly. Generally heat and bring to boil. Autoclave for 15 min at 121°C. Cool to 45-50°C. Sterilize skim milk powder by filtration Aseptically add 100 mL of cooled, sterilize skim milk solution. Mix thoroughly. Pour into sterilized petric dishes.

Protein-utilizing bacteria appear as colonies surrounded by a clear zone.

4. Starch agar (Ronald, 1992)

Composition (per liter)

Agar	20.0 g
Soluble starch	10.0 g
Gelatin	5.0 g
Beef extract	3.0 g
pH	6.8 ± 0.2

Preparation of agar

Add components to distilled water and bring volume to one liter. Mix thoroughly. Generally heat and bring to boiling. Autoclave for 15 min at 121°C. Pour into sterilized petri dishes.

After incubation, starch hydrolysis is determined by addition of Gram's iodine solution. Starch utilizing bacteria appear as colonies surrounded by a clear zone instead of blue background.

5. Tributyrin agar (Ronald, 1992)

Composition (per liter)

Agar	20.0 g
Tributyrin (glyceryl tributyrate)	10.0 g
Peptone	5.0 g
Yeast extract	3.0 g
pH	7.5± 0.2

Preparation of agar

Add components to distilled water and bring volume to one liter. Mix thoroughly. Generally heat and bring to boiling. Autoclave for 15 min at 121°C. Pour into sterilized petri dishes.

Lipid-utilizing bacteria appear as colonies surrounded by a clear zone.

APPENDIX 2 PREPARATION OF BRISTOL'S MEDIUM (Starr, 1960)

Preparation of stock solutions

$NaNO_3$	25 g/L
$CaCl_2$	2.5 g/L
$MgSO_4 \cdot 7H_2O$	7.5 g/L
K_2HPO_4	7.5 g/L
KH_2PO_4	17.5 g/L
NaCl	2.5 g/L

10 mL of each stock solution are added to 940 mL distilled water. Add a drop of 1% $FeCl_3$ solution to the mixture.

APPENDIX 3 ENUMERATION OF AMMONIA OXIDIZER BY MOST PROBABLE NUMBER METHOD (Schmidt and Balser, 1982)

Materials

1. Ammonium oxidizer medium

stock solution	**stock solution required per liter**
5% $(NH_4)_2SO_4$	10 mL
1.34% $CaCl_2 \cdot 2_H2O$	1 mL
4% $MgSO_4 \cdot 7H_2O$	1 mL
0.04% Bromothymol blue	5 mL
2.72% KH_2PO_4	7.5 mL
Chelated iron	1 mL
0.246% $FeSO_4 \cdot 7H_2O$	
0.331% EDTA disodium salt	
Trace element	1 mL
0.01 % $NaMoO_4 \cdot 2H_2O$	
0.02% $MnCl_2$	
0.0002% $CoCl_2 \cdot 6H_2O$	
0.01% $ZnSO_4 \cdot 7H_2O$	
0.002% $CuSO_4 \cdot 5H_2O$	

Add stock solution into about 800 mL distilled water. Adjust pH until blue color is formed by 1 N NaOH. Adjust volume to 1 L. Add 4 mL of this medium to each culture tube, cap the tubes, and sterilize for 15 min at 121°C.

2. Dilution water

stock solution	**stock solution required per liter**
2.72% KH_2PO_4	1 mL
3.84% K_2HPO_4	4 mL

Place 45 mL dilution into 50 mL screw-cup bottle. Sterilize this medium for 15 min at 121°C.

3. Modified Griess-Ilosvay reagents
   a. Diazotizing reagent
      Dissolve 0.5 g of sulfanilamide in 100 mL of 2.4 N hydrochloric acid. Store in refrigerator.
   b. Coupling reagent
      Dissolve 0.3 g of N-(1-naphthyl)-ethylenediamine hydrochloride in 100 mL of 0.12 N hydrochloric acid. Store solution in an amber bottle in refrigerator.

Procedures

Prepare a serial of 10-fold dilutions. Transfer 1 mL aliquots to each of five culture tubes. Incubate at 25-30°C in the dark.

Make initial observations after 3 weeks. Continue the incubation with weekly monitoring for at least 6 weeks or until there is no change in the number of positive tube for two consecutive weeks.

Both color change and presence of nitrite are employed to define positive result. Color change of medium from blue green to yellow indicates active acid production during oxidation of NH_4^+ to NO_2^-. Transfer a 0.1 mL aliquot aseptically to a spot plate, add 1 drop of diazotizing reagent and then 1 drop of coupling reagent. Color production (pink) indicates the presence of NO_2^-.

Record the number of positive tube, and estimate the ammonia oxidizing population using following table. p_1 is the number of positive tube in the least concentrated dilution in which all tubes are positive or in which the greatest number of tubes is positive. p_2 and p_3 represent the number of positive in the next two higher dilutions. Multiply the figure by the dilution factor of second dilution, i.e. dilution that obtains p_2 value.

Table of most probable numbers for use with 10-fold dilution and 5 tubes per dilution

		p3					
p1	p2	0	1	2	3	4	5
0	0	-	0.018	0.036	0.054	0.072	0.090
0	1	0.018	0.036	0.055	0.073	0.091	0.11
0	2	0.037	0.055	0.074	0.092	0.11	0.13
0	3	0.056	0.074	0.093	0.11	0.13	0.15
0	4	0.075	0.094	0.11	0.13	0.15	0.17
0	5	0.094	0.11	0.13	0.15	0.17	0.19
1	0	0.020	0.040	0.060	0.080	0.10	0.12
1	1	0.040	0.061	0.081	0.10	0.12	0.14
1	2	0.061	0.082	0.10	0.12	0.15	0.17
1	3	0.083	0.10	0.13	0.15	0.17	0.19
1	4	0.11	0.13	0.15	0.17	0.19	0.22
1	5	0.13	0.15	0.17	0.19	0.22	0.24
2	0	0.045	0.068	0.091	0.12	0.14	0.16
2	1	0.068	0.092	0.12	0.14	0.17	0.19
2	2	0.093	0.12	0.14	0.17	0.19	0.22
2	3	0.12	0.14	0.17	0.19	0.22	0.25
2	4	0.15	0.17	0.20	0.23	0.25	0.28
2	5	0.17	0.20	0.23	0.26	0.29	0.32
3	0	0.078	0.11	0.13	0.16	0.20	0.23
3	1	0.11	0.14	0.17	0.20	0.23	0.27
3	2	0.14	0.17	0.20	0.24	0.27	0.31
3	3	0.17	0.21	0.24	0.28	0.31	0.35
3	4	0.21	0.24	0.28	0.32	0.36	0.40
3	5	0.25	0.29	0.32	0.37	0.41	0.45
4	0	0.13	0.17	0.21	0.25	0.30	0.36
4	1	0.17	0.21	0.26	0.31	0.36	0.42
4	2	0.22	0.26	0.32	0.38	0.44	0.50
4	3	0.27	0.33	0.39	0.45	0.52	0.59
4	4	0.34	0.40	0.47	0.54	0.62	0.69
4	5	0.41	0.48	0.56	0.64	0.72	0.81
5	0	0.23	0.31	0.43	0.58	0.76	0.95
5	1	0.33	0.46	0.64	0.84	1.1	1.3
5	2	0.49	0.70	0.95	1.2	1.5	1.8
5	3	0.79	1.1	1.4	1.8	2.1	2.5
5	4	1.3	1.7	2.2	2.8	3.5	4.3
5	5	2.4	3.5	5.4	9.2	16	-

APPENDIX 4 ENUMERATION OF NITRITE OXIDIZER BY MOST PROBABLE NUMBER METHOD (Schmidt and Balser, 1982)

Materials

1. Nitrite oxidizer medium

stock solution	**stock solution required per liter**
0.85% KNO_2	1 mL
1.34% $CaCl_2 \cdot 2H_2O$	1 mL
4% $MgSO_4 \cdot 7H_2O$	5 mL
2.72% KH_2PO_4	1 mL
3.84% K_2HPO_4	4 mL
Chelated iron	1 mL
0.246% $FeSO_4 \cdot 7H_2O$	
0.331% EDTA disodium salt	
Trace element	1 mL
0.01 % $NaMoO_4 \cdot 2H_2O$	
0.02% $MnCl_2$	
0.0002% $CoCl_2 \cdot 6H_2O$	
0.01% $ZnSO_4 \cdot 7H_2O$	
0.002% $CuSO_4 \cdot 5H_2O$	

Add stock solution into about 800 mL distilled water. Adjust volume to 1 L. Add 4 mL of this medium to each culture tube, cap the tubes, and sterilize for 15 min at 121°C.

2. Dilution water (same as those for ammonia oxidizer)

3. Modified Griess-Ilosvay reagents (same as those for ammonia oxidizer)

Procedures

Prepare a serial of 10-fold dilutions. Transfer 1 mL aliquots to each of five culture tubes. Incubate at 25-30°C in the dark.

Make initial observations after 3 weeks. Continue the incubation with weekly monitoring for at least 6 weeks or until there is no change in the number of positive tube for two consecutive weeks.

Disappearance of NO_2^- is employed to define positive tube. Record the number of positive tubes in each of the dilutions and estimate the nitrite oxidizer population using above table.